Environmental Mass Spectrometry: Emerging Contaminants and Current Issues

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Review Contents

This biennial review covers developments in environmental mass spectrometry over the period of 2004−2005. A few significant references that appeared between January and February 2006 are also included. *Analytical Chemistry's* current policy is to limit reviews to include 100−200 significant references and to mainly focus on new trends. As a result, as was done in the previous 2004 Environmental Mass Spectrometry review (*1*), this 2006 review will be limited in its focus to new, emerging contaminants and environmental issues that are driving most of the current research. Even with a more narrow focus, only a small fraction of the quality research publications could be discussed. Thus, this review will not be comprehensive, but will highlight new areas and discuss representative papers in the areas of focus. I welcome any comments you have, in particular regarding this more narrow focus, whether you find it more (or less) useful than a broader approach (richardson.susan@epa.gov).

Numerous abstracts were consulted before choosing the best ones to present here. Abstract searches were carried out using *Current Contents*, and in many cases, full articles were obtained.

The overall trends in analytical methods include an increasing use of time-of-flight (TOF)-mass spectrometry (MS) and quadrupole (Q)-TOF-MS for structural elucidation and compound confirmation. TOF-MS and Q-TOF-MS provide increased resolution capability (typically 10 000−12 000 resolution), which allows precise empirical formula assignments for unknowns and also provides added confidence for positive identifications in quantitative work. This benefit of TOF-MS and Q-TOF-MS can be seen particularly in the sections on algal toxins and pesticide degradation products. On-line preconcentration methods, typically with on-line solid-phase extraction (SPE) or solid-phase microextraction

(SPME), are also becoming increasingly popular, as they allow methods to be more automated, more rapid, and reduce error in quantitative experiments. Large-volume injection is also becoming more popular, as more commercial instruments accommodate this technique, and it can allow lower detection limits due to the larger sample volume that can be injected. The last two years have also seen increased applications of hydrophilic interaction chromatography (HILIC) (usually coupled to liquid chromatography (LC)/ MS or LC/MS/MS), which uses columns with a polar end group (such as an amino group), and retention is based on the affinity of the polar analyte for the charged end group of the column stationary phase. This technique is ideal for separating highly polar analytes that are difficult to separate from the aqueous solvent front. As an example, HILIC has been shown to be useful for separating haloacetic acid disinfection byproducts (DBPs) for LC/ MS/MS analysis (see drinking water disinfection byproducts section for details). Previous methods have used ion pairing reagents to separate the polar analytes from the aqueous solvent front in LC, but ion pairing reagents can often suppress the ionization of analytes of interest. HILIC allows the separation of polar analytes and does not cause ionization suppression.

Trends in new environmental mass spectrometry research studies include increasing numbers of exposure and forensic studies. For example, there are interesting forensic investigations of algal toxins involved in dog poisoning episodes, where mass spectrometry was used to identify the algal toxins involved in these incidents. Interesting human exposure studies include the use of LC/electrospray ionization (ESI)-MS/MS to estimate exposure to organophosphorus pesticides by measuring their metabolites in urine. Another fascinating study involved measuring perfluorooctanoic acid (PFOA) in food packaging where perfluorinated chemicals (PFCs) are commonly used. Of the different food packagings investigated (including hamburger wrappers, french fry boxes, paper plates, dental floss, etc.), microwave popcorn bags were shown to leach extremely high levels of PFOA into the popcorn, such that consuming one bag of popcorn per month could account for as much as 20% of the average levels of PFOA found in human blood (see section on PFOA, perfluorooctanesulfonate (PFOS), and perfluorinated surfactants for more details). In addition, many of the new exposure studies are focusing on chiral contaminants, where enantiomer-specific uptake and metabolism can occur. For example, papers include the measurement of chiral toxaphene congeners in eggs from laying hens and the enantioselective elimination of toxaphene metabolites in fish.

As water is becoming more scarce in different regions of the United States, there has been a substantial increase in the number of studies involving reclaimed water. Examples include studies

involving the measurement of pharmaceuticals and DBPs in reclaimed water. In addition, as degradation products of pesticides have become increasingly important, pharmaceutical degradates (also called metabolites) are also becoming important. Improved analytical techniques are enabling their identification and measurement in environmental samples, and new studies address their fate in wastewater and drinking water treatment.

Six new categories of emerging contaminants are added to this environmental mass spectrometry review this year: new brominated/chlorinated flame retardants (beyond polybrominated diphenyl ethers (PBDEs)), sunscreens/UV filters, contaminant-DBPs, benzotriazoles, naphthenic acids, and arsenic mode of action studies. Interesting new research by Hites' group has uncovered new brominated/chlorinated flame retardants that were not previously known. One of these, Dechlorane Plus (bis-(hexachlorocyclopentadieno)cyclooctane) has been on the market for more than 40 years but was just identified in environmental samples this year. Two other brominated flame retardants, 1,2-bis(2,4,6-tribromophenoxy)ethane (TBE) and 2,3,4,5,6-pentabromoethylbenzene (PEB), were also reported for the first time in air samples, and they can be present at higher levels than the commonly observed PBDEs. Flame retardants are widely used in products such as furniture, textiles (including children's clothing), plastics, paints, and electronic appliances (including most computers), and they have been found to be environmentally persistent. UV filters are widely used in products such as sunscreens, cosmetics, beauty creams, skin lotions, hair sprays, hair dyes, and shampoos, and their analysis in environmental samples has increased substantially the last two years, so this new category of emerging contaminant is included in this review for the first time. While drinking water DBPs have been an issue for several years (and new emerging DBPs have recently become important), reaction products of contaminants (such as pesticides, antibacterial agents, estrogens, alkylphenol ethoxylates, and cyanobacterial toxins) with disinfectants such as chlorine or ozone are now being investigated. Benzotriazoles, which are widely used as anticorrosives and for silver protection in dishwashing liquids, are becoming important, emerging environmental contaminants because they are environmentally persistent and resistant to wastewater treatment. Naphthenic acids are surfactant-like contaminants that result from the extraction of crude oil from oil sands regions. They are highly toxic to aquatic organisms, and they can be found up to 100 mg/L in tailing pond waters. Studies of naphthenic acids have increased in the last two years. They are an analytical challenge because of the large number of congeners and the difficulty in precisely identifying individual isomers. Finally, mass spectrometry is being used to conduct exciting new biological studies of arsenic to understand the mode of action of arsenic and its metabolism. Previous environmental mass spectrometry reviews (2002 and earlier) and water analysis reviews have focused on arsenic speciation and new analytical methods, but this is the first time that biological studies for understanding the health effects of arsenic have been covered in this review.

Other emerging contaminants again include perfluorinated surfactants (including PFOS and perfluorooctanoic acid (PFOA)), PBDEs, pharmaceuticals, hormones, endocrine disrupting compounds (EDCs), DBPs, algal toxins, perchlorate, pesticide degradation products, chiral contaminants, and microorganisms.

These continue to be intense areas of research. The class of emerging contaminant experiencing the most rapid growth in research is the perfluorinated surfactants. They were included for the first time in the last environmental mass spectrometry review (2004), and as studies continue to show their widespread presence in human blood and biota, and health effects issues with them are uncovered, they remain a high priority for the U.S. Environmental Protection Agency (EPA).

GENERAL REVIEWS

This section includes general reviews relating to environmental mass spectrometry. Reviews that relate to specific areas (e.g., pharmaceuticals, DBPs, or microorganisms) can be found in those specific sections. Many reviews have been published over the last two years that relate to environmental mass spectrometry, and a few focus specifically on emerging contaminants. The previous biennial environmental mass spectrometry review published in 2004 contained 200 references and discussed advances in research for emerging contaminants and issues, including, PFOS and PFOA, pharmaceuticals, hormones, endocrine disruptors, drinking water DBPs, chemical warfare agents, chiral contaminants, algal toxins, pesticide degradation products, alkylphenol ethoxylate surfactants, organotins, perchlorate, methyl *tert*-butyl ether, arsenic, microorganisms, and natural organic matter (*1*). This review covered developments from 2002 to 2003.

A biennial review on water analysis published in 2005, included a discussion of emerging contaminants and current issues that are important for water, as well as a discussion of new regulations and regulatory methods that have been developed (*2*). This water analysis review covered developments from 2003 to 2004.

Emerging contaminants were also the focus of a recent book. *Emerging Organic Pollutants in Wastewaters and Sludge* includes discussions about pharmaceuticals, surfactants, estrogenic compounds, and methods for emerging industrial pollutants (*3*). Koester published a nice biennial review on trends in environmental analysis in 2005 (*4*). In this review, Koester discusses developments in sample collection and extraction methods (including semipermeable membrane devices, SPME, hollow fiber-liquid phase microextraction, and SPE); separation and detection techniques (including new stationary phases, chiral separations, two-dimensional GC, mass spectrometry, and nuclear magnetic resonance (NMR)); and emerging detection techniques (including accelerator mass spectrometry and high-field asymmetric waveform ion mobility spectrometry (FAIMS)). Koester also discusses analytes of emerging interest, whose analysis is made possible by recently developed analytical instruments and methods. This review is a must-read for anyone wanting an update on analytical developments for environmental analysis.

Vas and Vekey published a review on the use of SPME prior to MS analysis (*5*) and discuss specific environmental applications. Guevremont reviewed the use of FAIMS with mass spectrometry, covering the fundamentals of FAIMS technology and applications to environmental analysis (*6*). Some of the benefits of FAIMS include the separation of isobaric ions (e.g., diastereomers), separation of isotopes, and reduction of chemical noise in ESI-MS applications.

Schmitt-Kopplin and Englmann reviewed major developments and applications of capillary electrophoresis (CE)-MS, which

included environmental applications (7). Schmidt et al. published a review on the state-of-the-art, prospects, and future challenges of compound-specific stable isotope analysis, using GC/isotope ratio-MS (8). Discussed in this review are the determination of contaminant sources on a local, regional, and global scale; the identification and quantification of transformation reactions; and the characterization of reaction mechanisms. Two reviews focused on inductively coupled plasma (ICP)-MS. First, Rosen and Hieftje published a review on the use of ICPMS and ESI-MS for elemental speciation analysis (9). In this review, the authors discuss current areas of research and instrumentation used to solve speciation problems. In the second review, Becker covered the use of ICPMS and laser ablation ICPMS for isotope analysis of long-lived radionuclides in environmental and nuclear waste materials (10). Finally, Butler et al. published a review on the application of atomic spectrometry for the analysis of environmental samples (11).

PFOA, PFOS, AND OTHER PERFLUORINATED SURFACTANTS

Perfluorinated surfactants (also referred to as fluorotelomer acids, alcohols, and sulfonates) have been manufactured for more than 50 years and have been used to make grease/stain repellents (such as Teflon) that are widely applied to fabrics, carpets, cookware, and paper (12). They are also used in the manufacture of many products, including paints, adhesives, waxes, polishes, metals, electronics, and caulks, as well as grease-proof coatings for food packaging (e.g., microwave popcorn bags, french fry boxes, hamburger wrappers, etc.). During 2000−2002, an estimated 5 million kg/yr of these compounds was produced worldwide, with 40% of this in North America. Two of these fluorinated surfactants, perfluorooctanesulfonate and perfluorooctanoic acid are currently receiving a great deal of attention as emerging contaminants in the United States. PFOS was once used to make the popular Scotchgard fabric and carpet protector, and since 2002, it is no longer manufactured due to concerns about widespread global distribution in the blood of the general population and in wildlife, including remote locations in the Arctic and North Pacific Oceans. However, other fluorinated surfactants such as PFOA are still manufactured and are used to make soil, stain, grease, and water-resistant coatings. Like PFOS, PFOA appears to be ubiquitous at low levels in human blood, even in those people living far from any obvious sources. Most Americans have about 4−5 ppb PFOA in their blood (www.epa.gov/oppt/pfoa/pfoarisk.htm). Research questions include understanding the sources of PFOA and other fluorinated surfactants, their environmental fate and transport, pathways for human exposure and uptake, and potential health effects. Scientists in academia, industry, and the U.S EPA have launched investigations to tackle these questions. In addition, the Centers for Disease Control and Prevention (CDC) has recently nominated PFOS and PFOA to be included in their National Health and Nutrition Examination Survey (NHANES) to provide a better assessment of the distribution of these chemicals in human populations (13).

In January 2005, the U.S. EPA issued a draft risk assessment on PFOA, which included an analysis of how PFOA causes liver tumors in rats and the relevance of this mode of action for human health risk assessment (www.epa.gov/oppt/pfoa/pfoarisk.htm). While previous studies have shown that PFOA can cause cancer in animals (liver, testicular, and pancreatic), there are questions regarding the relevance of these animal results to humans (14). A preliminary epidemiologic investigation of workers at a plant occupationally exposed to PFOA and residents living near this plant is indicating the possibility of elevated rates for prostate cancer in young men and uterine cancer in women, along with uncommon cancers, such as non-Hodgkin's lymphoma, leukemia, and multiple myeloma (12). This plant is also conducting its own survey of possible PFOA effects on 750 volunteer employees (12). There are also new studies into the possible developmental toxicity of PFOA and other fluorinated surfactants (13). PFCs are unusual chemically, in that they are both hydrophobic (repel water) and lipophobic (repel lipids/grease), and they contain one of the strongest chemical bonds (C−F) known. Because of these properties, they are highly stable in the environment (and in biological samples), and they are expected to have unique profiles of distribution in the body (13). While most of the current attention is on PFOA (which is generally the most commonly found fluoro acid), it is important to note that other higher chain fluoro acids (beyond C8) are also contained in these fluorotelomer products and have been found in human blood and other biological and environmental samples.

The U.S. EPA recently challenged eight chemical companies to eliminate PFOA and other PFCs from their products and facility emissions worldwide by 2015 (15). So far, two companies have agreed, including the only U.S. manufacturer of PFOA. This U.S. manufacturer recently agreed to fund projects to evaluate the potential for nine fluorotelomers to break down into PFOA (16).

LC/MS and LC/MS/MS are the most common analytical techniques used for the measurement of PFCs; however, there can be difficulty in obtaining clean analytical blanks, due to inherent contamination in LC systems (fluoropolymer coatings on seals, etc.). As a result, GC/MS and GC/MS/MS are sometimes used. In a nice systematic study, Yamashita et al. isolated and determined sources of PFC contamination for SPE-LC/MS measurements (17). Initially, solvent blanks (using a 10-μL injection of pesticide-grade methanol) were found to consistently contain significant PFOS and PFOA contamination when analyzed by LC/MS. PFOA was the most abundant contaminant, at 30 pg/10 μL injected. The LC tubing (made of poly(tetrafluoroethylene), PTFE), internal LC instrument parts (coatings and seals in the degasser and solvent selection valves), and autosampler vial septum were all found to be sources of the PFC contamination. As a result, the investigators replaced the LC tubing with polyetheretherketone and stainless steel tubing, replaced solvent inlet filters with stainless steel filters, and replaced Teflon or Viton autosampler vial caps with polyethylene, which decreased the instrumental blank contamination considerably. The authors also found that polypropylene sample bottles can contain up to 27 ng/L PFOA, and Sep-pak SPE cartridges can contain 46 and 12 pg/L PFOA and PFOS, respectively. By switching to an Oasis SPE cartridge (Waters, Milford, MA), levels of PFOA and PFOS were reduced by a factor of 10 and 5−10, respectively. Purified reagent water used for field blanks was also found to be contaminated with PFCs; however, Milli-Q and high-performance LC-grade water contained lower background levels than distilled water. Finally, nylon syringe filters that are used to remove particles from extracts prior to LC/MS/MS injection were also found to contain PFOA (25−75 pg) in three different brands

investigated, with PFOS also being detected in two of these brands. Washing the filters with methanol (2 mL) prior to filtration of the samples was found to eliminate the PFOA and PFOS residues. It is evident that in order to determine PFOA, PFOS, and other PFCs at low detection limits, considerable effort must be taken to ensure clean blanks.

Two reviews have been published the last two years on PFOS, PFOA, and other PFCs. Kennedy et al. wrote a review on the toxicology of PFOA, where they discuss the different mechanisms involved in the types of tumors observed in animal studies (*14*). Lau et al. reviewed the developmental toxicity of PFOA and PFOS (*13*). Issues involved in extrapolating animal data to humans are discussed, and future research and directions are outlined.

Several studies have recently focused on determining the sources of PFCs in humans and biota. In a fascinating study from Begley et al., potential sources from food packaging were investigated (*18*). Sources, such as PTFE (Teflon)-coated cookware, dental floss, PTFE film/sealant tape, fluoroethylene propene copolymer (FEP) tubing, microwave popcorn bags, hamburger wrappers, french fry boxes, and paper plates (coated with a soak-proof shield), were investigated for their PFOA content and the transfer of PFOA to foods. ESI-MS was used to measure PFOA (with a ^{13}C-labeled PFOA internal standard) and other PFCs; ESI-MS/MS (with multiple reaction monitoring (MRM)) was used for PFOA confirmation. Most products sampled contained low-microgram per kilogram levels of PFOA, and nonstick cookware was found to not be a major source of PFOA. However, some microwave popcorn bags were shown to leach as high as 300 μg/kg PFOA into popcorn, making this a major potential source of PFOA. In fact, it is estimated that eating one bag of popcorn per month for a year can account for as much as 20% of the average PFOA levels in the blood of U.S. residents. The reason for the high level of PFOA migration to the popcorn was attributed to rapid heating (to over 200 °C) and the presence of oil in the popcorn bag. However, this effect was not observed with all microwave popcorn brands, as some microwave popcorns had PFCs applied to the outside of the bag, rather than the inside. In another food migration study, Powley et al. measured the transfer of PFOA from nonstick frying pans coated with fluoropolymers under simulated cooking conditions (*19*). LC/MS/MS determinations showed that none of the cookware samples analyzed had detectable levels of PFOA. Detection and quantification limits of the method were 100 pg/cm^2.

New studies are also addressing the reasons behind the widespread detection of perfluorinated acids in the environment. In particular, it has been suggested that fluorinated telomer alcohols are probable precursor compounds that may undergo transformation reactions in the environment to form the fluorinated acids. As a result, Dinglasan et al. investigated the aerobic biodegradation of the 8:2 telomer alcohol ($CF_3(CF_2)_7CH_2CH_2OH$) (*20*). GC/MS was used to identify volatile metabolites, and LC/MS/MS was used to identify and quantify nonvolatile metabolites. In this study, PFOA and two other telomer acids [$CF_3(CF_2)_7CH_2COOH$ and $CF_3(CF_2)_6CFCHCOOH$] were found to be metabolites during the degradation. The overall mechanism involved the oxidation of the 8:2 fluorinated telomer alcohol to the fluoro acid via a transient telomer aldehyde. The fluoro acid was further transformed to the unsaturated acid, which ultimately formed the

highly stable PFOA. It was suggested that biological transformation may be a major degradation pathway for fluorinated telomer alcohols in aquatic systems. In related work, Ellis et al. used smog chamber studies to study the atmospheric degradation of fluorotelomer alcohols to fluoro acids (*21*). GC/MS (with derivatization) and LC/MS/MS were used for identification of degradates formed. In this study, the fluorotelomer alcohols were found to degrade to form PFOA and other fluorinated acids, and the fluoro acids showed distinctive profiles that are also observed in arctic animals. It was concluded that atmospheric degradation of the fluoro alcohols is likely to contribute to the widespread distribution of the fluoro acids, including PFOA. In another study of atmospheric transformations, perfluoroalkanesulfonamides were investigated as a potential source of fluoro acids. Martin et al. used LC/MS/MS in smog chamber experiments to investigate these transformations (*22*). Results showed that fluoro acids (including 2-, 3-, and 4-carbon fluroacids) did form from the sulfonamides, via the formation of aldehyde and ketone intermediates.

Wastewater and sludge have also been investigated as sources of PFOA and other PFCs. In one study, Boulanger et al. used LC/ESI-MS/MS to measure PFOA, PFOS, and six other PFCs in wastewater that had no known manufacturing or industrial application of PFCs (*23*). 2-(*N*-Ethylperfluorooctanesulfonamido) acetic acid (*N*-EtFOSAA) was found in influent (5.1 ng/L), effluent (3.6 ng/L), and river water samples (1.2 ng/L); PFOS and PFOA were found in effluent (26 and 22 ng/L, respectively) and river water (23 and 8.7 ng/L, respectively). Further biotransformation studies indicated that the transformation of precursors in wastewater treatment is not an important source of these PFCs. Results suggested that direct use and disposal of products containing the end products were likely to be a more important source. In another more extensive study of wastewater, Schultz et al. investigated the occurrence and transformation of PFCs at 10 wastewater treatment plants in different regions of the United States (*24*). Their analytical method utilized centrifugation followed by large-volume injection (500 μL) of the supernatant onto a reversed-phase LC column and detection using ESI-MS/MS. Perfluoroalkyl sulfonates, fluorotelomer sulfonates, perfluorocarboxylates, and fluorinated alkyl sulfonamides were measured in this study. PFCs were found in wastewater from all treatment plants, and at least one class of PFC increased in concentration after treatment in most plants sampled. In some cases, decreased levels of certain PFCs were observed, which was attributed to sorption onto the sludge. In addition, each wastewater treatment plant exhibited a unique fingerprint of PFCs, despite very similar treatment processes. In another study, Higgins et al. used LC/MS/MS to investigate the concentrations of PFCs in sludge resulting from wastewater treatment at several locations in the San Francisco Bay area (*25*). Liquid solvent extraction was used for this analytical method, followed by SPE cleanup, and injection of extracts with internal standards. Detection limits ranged from 0.7 to 2.2 and 0.041 to 0.246 ng/g (dry weight) for sludge, and sediment, respectively. The concentrations of PFCs in domestic sludge ranged from 5 to 152 ng/g for total perfluorocarboxylates and 55 to 3370 ng/g for total perfluoroalkyl sulfonyl-based chemicals. Highest levels of PFOS and PFOA were 2610 and 13.3 ng/g, respectively. Data from a survey of San Francisco Bay area sediments showed widespread occurrence of PFCs in sediments

at low-nanogram to subnanogram per gram levels. In addition, substances that might be transformed to PFOS (such as *N*-EtFOSAA and 2-(*N*-methylperfluorooctanesulfonamido) acetic acid (*N*-MeFOSAA)) were present in both sediments and sludge, at levels often exceeding PFOS.

Groundwater was the focus of another study. Schultz et al. used a direct injection-LC/ESI-MS/MS method to measure fluorotelomer sulfonates in groundwater collected from U.S. military bases where PFCs had been used in fire-fighting foams during military exercises (*26*). This method allowed detection down to 0.60 µg/L and was used to measure 11 PFCs, including PFOS, PFOA, perfluorobutanesulfonate (PFBS), and perfluorohexanesulfonate (PFHS). Total fluorotelomer sulfonate concentrations ranged from below quantitation to 14 600 µg/L. A separate analysis of this fire-fighting foam revealed only small amounts of fluoroteomer sulfonates, but substantial contribution from fluoroalkylthioamido sulfonates.

Surface water studies of PFCs were conducted in Japan and in the United States. Saito et al. measured PFOA and PFOS in Japanese surface waters using a SPE-LC/MS method, which could achieve 0.06 and 0.04 ng/L detection limits, respectively (*27*). The highest mean concentrations reached 21.5 and 5.73 ng/L for PFOA and PFOS, respectively, and systematic searches revealed a major point-source contamination of PFOA from a public water disposal site. An airport was a major source of contamination of PFOS. Great Lakes waters were the focus of another study. Boulanger et al. used LC/MS and LC/MS/MS to measure eight perfluorooctane surfactants, including PFOA, PFOS, and the PFOS precursors, perfluorooctanesulfonylamide (FOSA), *N*-ethylperfluorooctane sulfonamidoethanol (*N*-EtFOSE), *N*-ethylperfluorooctane sulfonamide (*N*-EtFOSAA), 2-(perfluorooctanesulfonamido)-acetic acid (PFOSAA), and perfluorooctane sulfinate (PFOSulfinate) in Lake Erie and Lake Ontario (*28*). This study represents the first measurement of PFOS precursors in any water body. PFOS and PFOA levels ranged from 21 to 70 and 27 to 50 ng/L, respectively. *N*-EtFOSAA, FOSA, perfluorooctanesulfonylamide (PFOSA), and PFOSulfinate were found in several samplings at 4.2−11, 0.6−1.3, and <2.2−17 ng/L, respectively.

PFCs have also been measured in seawater. Yamashita used LC/ESI-MS/MS to carry out a global survey of PFOS, PFOA, PFHS, PFBS, perfluorononanoic acid (PFNA), and PFOSA in marine samples (*29*). This paper also provides a nice summary of PFOS and PFOA measurements in the livers of various marine mammals and birds, as well as toxicity effects of PFOS in aquatic organisms. Seawater is a particularly challenging matrix for PFC measurements because of the lower levels (pg/L, parts-per-quadrillion) of PFCs in seawater. This method used Oasis HLB cartridges for extraction of the fluoro acids from seawater, which gave lower background levels and greater recoveries than other SPE cartridges. Method detection limits were in the low picogram per liter range, and this method was used to measure these PFCs at 19 locations in the Pacific Ocean, 5 locations in the South China Sea and Sulu Sea, 12 locations in the mid-Atlantic Ocean, and 20 locations in the Labrador Sea. PFOS and PFOA were found in 80% of the seawater samples analyzed. Tokyo Bay contained the highest levels of PFOA (192.0 ng/L) and PFOS (57.7 ng/L); levels in the open oceans were lower, in the picogram per liter range. The higher levels of PFOA relative to PFOS in the seawater,

coupled with previous findings of higher levels of PFOS in wildlife, suggest that PFOS is more bioaccumulative than PFOA.

Several studies have focused on the measurement of PFCs in biological samples. In one of the largest studies of this kind, Kannan et al. used LC/ESI-MS/MS to measure PFOS, PFHS, PFOA, and PFOSA in 473 human blood/serum/plasma samples collected from the United States, Colombia, Brazil, Belgium, Italy, Poland, India, Malaysia, and Korea (*30*). PFOS was the most predominant PFC found in blood and was highest in blood samples collected from the United States and Poland (up to 164 ng/mL). Levels were moderate in Korea, Belgium, Malaysia, Brazil, Italy, and Colombia (3−29 ng/mL), and lowest in India (<3 ng/mL). PFOA was the next most abundant PFC, which reached a maximum of 256 ng/mL. No age- or gender-related difference was observed, and the proportions of the PFCs varied by location, suggesting the existence of sources with varying levels and compositions and differences in exposure patterns to these chemicals in different countries. In another study of PFCs in human blood, Yeung et al. did observe gender-related differences in the concentration profile of PFOA, PFOS, PFOSA, and PFHS in blood samples from nine cities in China (*31*). Males had higher levels of PFOS and PFHS, and females had higher levels of perfluoroundecanoic acid. This is also one of the few studies to measure PFCs with higher carbon chains. Liquid−liquid extraction was used to extract the PFCs from blood, and ESI-MS/MS was used for detection. Of the PFCs measured, PFOS was predominant.

Human maternal and cord blood was the focus of another study by Inoue et al. (*32*). In this study, on-line extraction with LC/ESI-MS was used to measure PFOS, PFOA, and PFOSA, with detection limits of 0.5, 0.5, and 1.0 ng/mL, respectively. Pregnant women, 17−37 years old, were enrolled in this study. PFOS concentrations in maternal blood ranged from 4.9 to 17.6 ng/mL, while fetal samples ranged from 1.6 to 5.3 ng/mL. PFOA was detected only in maternal blood samples (up to 2.3 ng/mL); PFOSA was not detected in either type of sample. A high correlation was found between PFOS concentrations in maternal blood and cord blood. This study revealed that human fetuses are exposed to PFCs.

Several new LC/MS methods have been developed to measure PFCs in biological samples. A new packed capillary LC/ESI-ion trap-MS method was developed by Holm et al. to measure PFOA and PFOS in human plasma (*33*). Analyses required only 10 min, and detection limits of 0.5 and 0.2 ng/mL were achieved for PFOA and PFOS, respectively. A similar method using SPE with column-switching LC/ESI-MS was developed by Inoue et al. to measure PFOA, PFOS, and PFOSA in human plasma (*34*). Detection limits were 0.5, 0.5, and 1.0 ng/mL, respectively. Flaherty et al. developed a clever method using acetonitrile extraction in a 96-well plate format to extract PFOA from serum and plasma samples (*35*). This method enables the rapid analysis of large numbers of samples; LC/ESI-MS/MS was used for detection, and detection limits of 0.5 mg/mL were obtained. Tseng et al. developed a new LC/ESI-ion trap-MS method to measure PFOA, PFOS, and perfluorodecanoic acid (PFDA) in water and biological tissues (fish and oysters) (*36*). Quantification limits were 0.5−6 ng/L for water and 5−50 ng/g for biological tissues.

Several interesting PFC studies have also been carried out in fish, Arctic mammals, and aquatic organisms. Falandysz et al. used LC/ESI-MS to measure 19 PFCs in fish, duck, and human blood from the Gulf of Gdansk region of Poland (37). This study revealed that, in addition to PFOS and PFOA, 8 additional PFCs bioaccumulated in the human body and that the food chain is an important route of exposure for all 10 of the PFCs detected. Individuals who ate the most fish contained on average the highest levels of PFCs. PFCs measured in this study include PFOA, PFOS, PFHxA, PFOSA, PFHS, perfluoroheptanoic acid, PFNA, PFDA, PFUnDA, and perfluorododecanoic acid. In another extensive study, Tomy et al. used LC/ESI-MS/MS to measure four PFCs (PFOS, PFOA, PFOSA, N-EtPFOSA) in an eastern Arctic marine food web (beluga whale, narwhal, walrus, redfish, birds, fish, shrimp, and zooplankton) (38). This study is the first to measure N-EtPFOSA, which was found in all species except redfish, at mean concentrations ranging from 0.39 to 92.8 ng/g. PFOS was detected in all species analyzed, at mean concentrations ranging from 0.28 to 20.2 ng/g; PFOA was detected in 40% of the samples at concentrations generally less than PFOA, with the greatest concentrations in zooplankton (2.6 ng/g). PFOSA was only detected in the livers of beluga whales and narwhals. Biomagnification estimates for PFOS ranged from 0.4 to 9. Results showed that PFOS biomagnifies in the Arctic marine food web.

Another study by Keller et al. focused on PFC measurement in sea turtles (39). LC/ESI-MS/MS was used to measure 12 PFCs in the plasma of 73 loggerhead sea turtles and 6 Kemp's Ridley sea turtles captured from in shore waters of Core Sound, NC, and offshore waters of South Carolina, Georgia, and Florida. PFOS and PFOA were the dominant PFCs found, with mean concentrations of 11.0 and 3.20 ng/mL, respectively, for loggerhead turtles, and 39.4 and 3.57 ng/mL, respectively, for Kemp's Ridley turtles. Mean PFOS concentrations were 2−12-fold higher than typical mean polychlorinated biphenyl (PCB) concentrations measured previously in sea turtle blood. More than 79% of the samples contained detectable levels of perfluorocarboxylates with 8−12 carbons, whereas only 17% or less of the samples had detectable levels of perfluorocarboxylates with 6 or 7 carbons, and no samples had perfluorocarboxylates with 4 or 5 carbons. Finally, Smithwick et al. used LC/ESI-MS/MS to measure 12 PFCs in liver tissue from east Greenland polar bears (40). This was the largest study to-date to investigate PFCs in polar bears. PFCs measured included PFOS, PFOA, PFOSA, PFHS, and perfluorocarboxylates with 9, 10, 11, 12, 13, 14, and 15 carbons. Concentrations of PFOS (mean of 2470 ng/g) were similar to levels found in polar bears from Hudson Bay, Canada, and both populations had significantly higher concentrations than reported in Alaska, which suggested a special trend.

PHARMACEUTICALS, HORMONES, AND ENDOCRINE DISRUPTING COMPOUNDS

Pharmaceuticals, hormones, and EDCs have become major issues in environmental chemistry, due to their presence in environmental waters (following incomplete removal in wastewater treatment or point-source contaminations), threat to drinking water sources, and concern about possible estrogenic and other effects, both to wildlife and to humans. A major concern for pharmaceuticals also includes the development of bacterial resistance (creation of "Super Bugs") from the release of antibiotics in the environment. It is estimated that ∼3000 different substances are used as pharmaceutical ingredients today, including painkillers, antibiotics, antidiabetics, β-blockers, contraceptives, lipid regulators, antidepressants, and impotence drugs (2). However, only a very small subset of these compounds (∼150) have been investigated in environmental matrixes (2). Pharmaceuticals are introduced not only by humans but also through veterinary use in livestock, poultry, and fish farming. Various drugs are commonly given to farm animals to prevent illness and disease and to increase the size of the animals. There has been uncertainty about whether the low environmental levels of pharmaceuticals (generally ng/L) would cause adverse effects on humans or wildlife. While health effects studies are still limited, estrogenic effects (2) and renal effects (41) have been reported for α-ethinylestradiol and diclofenac, respectively, at low environmentally relevant concentrations. However, a recently published human health risk assessment for 26 active pharmaceutical ingredients and their metabolites (representing 14 different drug classes) predicts that there would be no appreciable human health risk from the presence of these 26 particular compounds at trace concentrations in surface water or drinking water (42). There is still a scarcity of human risk assessments for environmental exposure to pharmaceuticals, though, so it is premature to draw firm conclusions at this time and to extrapolate this limited assessment to pharmaceuticals beyond the 26 investigated.

Many pharmaceuticals, hormones, and EDCs are highly polar—which necessitates the use of either LC/MS (and LC/MS/MS) or an efficient derivatization procedure combined with GC/MS (and GC/MS/MS) for their analysis. These mass spectrometry methods can typically measure pharmaceuticals at low-nanogram per liter levels in environmental samples. ESI and atmospheric pressure chemical ionization (APCI) are the more commonly used LC interfaces, but atmospheric pressure photoionization (APPI) and sonic spray ionization are also now being used. Increasingly, tandem-MS and multiple reaction monitoring (MRM) are being used with both LC/MS and GC/MS to provide added selectivity and sensitivity. Innovations have also been made in rapid on-line extraction, microextraction, and on-line derivatization techniques used in combination with GC/MS/MS detection.

Pharmaceuticals. As more research groups are investigating pharmaceuticals in the environment, several reviews have been published recently. Petrovic et al. published a review on LC/tandem-MS for the analysis of pharmaceuticals in environmental samples (43). This review covers sample preparation procedures, chromatographic separation techniques, and MS detection techniques using triple quadrupole, TOF, and Q-TOF mass spectrometers. SPE is the most commonly used extraction technique, with C18 silica sorbents and polymeric Oasis HLB cartridges commonly used to extract and concentrate acidic nonsteroidal antiinflammatory drugs. Oasis MCS mixed sorbents, which have both cation-exchange and reversed-phase characteristics, are commonly used for polar to semipolar pharmaceuticals. Complete chromatographic separations using LC are beneficial to MS analyses because this generally improves detection and reduces ion suppression that can occur with ESI or APCI-MS. For MS detection, advantages and drawbacks were cited for the commonly used techniques (triple quadrupole, TOF, and Q-TOF-MS). Triple quadrupole-MS

remains the primary detection/quantification method of choice because of the selectivity and sensitivity afforded through MRM analyses, as well as the high dynamic range (>4 orders of magnitude). In contrast, TOF-MS and Q-TOF-MS can offer an additional advantage of providing exact mass data (resolutions of 10 000−12 000 are common), which helps to ensure correct identifications and prevent false positives, but these instruments are more limited in their dynamic range (usually 2 or 3 orders of magnitude). Several applications of these techniques to different classes of pharmaceuticals are also provided in this very nice review. In another review by Kim and Carlson, the use of LC/MS/MS for quantifying trace pharmaceuticals was discussed (44). This review also covers sources of pharmaceuticals and some occurrence information. Debska et al. reviewed the fate and analysis of pharmaceuticals in the aquatic environment, which covered the presence of metabolites in the environment, as well as input by different sources (45). Zuccato reviewed pharmaceuticals in environmental samples from Italy and discussed causes, occurrence, and control (46). Included in this review was a discussion of in vitro and in vivo studies that suggest pharmaceuticals may have ecotoxicological effects.

Several new analytical methods have been published in the last two years on the measurement of pharmaceutical compounds in surface water, groundwater, wastewater, and sludge. One of these methods developed by Hao et al. used a single-step SPE and LC/ESI-MS/MS to measure 27 antibiotics and neutral pharmaceuticals in aqueous environmental samples collected from rivers and streams in Ontario, Canada (47). Detection limits ranged from 20 to 1400 ng/L. This study reported the first finding of monensin in the environment. Cahill et al. published the SPE-LC/ESI-MS method that had been used in the previous U.S. Geological Survey (USGS) nationwide study of pharmaceuticals in U.S. surface water and groundwater (48). Twenty-two pharmaceuticals were included in this method, with average method detection limits of 0.022 μg/L. This previously published nationwide study revealed the widespread presence of pharmaceuticals routinely at 0.020−0.100 μg/L and at levels as high as 5.2 μg/L.

On-line SPE methods are also becoming more popular. Pozo et al. reported such a method using on-line SPE coupled to LC/ESI-MS/MS (triple quadrupole) to measure 16 antibiotics in water (49). Limits of detection were 0.4−4.3 ng/L. The use of Q-TOF-MS for confirming identifications was also discussed. Stoob et al. developed an automated on-line SPE-LC/ESI-MS/MS method using Oasis HLB SPE cartridges and column switching techniques to measure both sulfonamide antibiotics, their metabolites, and some pesticides (50). High sensitivity in the low nanogram per liter range was achieved by large-volume injections of 18 mL. This configuration allowed high sample throughput, and more than 500 samples could be analyzed with one extraction cartridge.

A few analytical methods developed avoid the use of preconcentration. For example an ion pair reversed-phase LC/ESI-MS/MS method was developed to measure 12 acidic pharmaceuticals (nonsteroidal antiinflammatory drugs and bezafibrate), 2 metabolites, and triclosan in surface water and wastewater (51). This method allows the analysis of wastewater samples without preconcentration. Tri-n-butylamine was used as the ion-pairing agent, and it increased the signal intensity for all of the acidic analytes. Detection limits of 6−200 ng/L were achieved. If SPE was used

with this method, lower limits of 0.15−11 ng/L could be obtained. Using this method, levels up to 5.5 μg/L were found in surface waters and wastewaters. van der Ven et al. also developed new method that does not require preconcentration using a capillary LC/ESI-MS/MS to measure diazepam in aquatic samples (52). Diazepam is prescribed as an anticonvulsant, hypnotic, anxiolytic, and muscle relaxant and is in one of the most used classes of drugs in the world. Detection limits of 0.1 μg/L were achieved, and the method was used to measure wastewater influent and effluent samples in Belgium.

Wastewater and biosolids were the focus of another LC/ESI-MS/MS method by Miao et al. (53). In this study, the antiepileptic drug, carbamazepine, its major metabolites, and caffeine were measured at different treatment stages of a wastewater treatment plant. Carbamazepine and its metabolites were found at levels ranging from 1.6 to 69.6 μg/kg (dry weight) in untreated biosolids and at concentrations ranging from 3.4 to 258.1 μg/kg (dry weight) in the treated biosolids. The results showed that 29% of the carbamazepine was removed from the aqueous phase, most likely from degradation. Therefore, the majority of carbamazepine and its metabolites exist in the aqueous phase (wastewater), rather than in the biosolids.

Antibiotics have been the focus of several studies in surface water and wastewater. In some cases, wastewater treatment did not provide complete removal of the antibiotics, allowing them to re-enter rivers from these discharges following treatment. Göbel et al. developed a SPE-LC/ESI-MS/MS method to measure four macrolide antibiotics, six sulfonamides, trimethoprim, and the human metabolite N-4-acetylsulfamethoxazole in wastewater (54). Using this method, the authors were able to find concentrations ranging from 10 to 423 ng/L in the tertiary effluents, which corresponded to 22−1450 ng/L in the primary effluents, indicating some (but not complete) elimination in wastewater treatment. Yang and Carlson developed a SPE-LC/ESI-ion trap-MS/MS method to measure the antibiotics—erythromycin, roxithromycin, and tylosin—in surface water and wastewater (55). Method detection limits were 0.07, 0.03, and 0.05 μg/L, respectively, and the method was used to measure these antibiotics in a river and wastewater treatment plant in Colorado. Antibiotics were only detected in the wastewater treatment plant and immediately downstream of the wastewater treatment plant. In another study, Lindberg et al. used SPE with LC/ESI-MS/MS to measure 12 antibiotics in wastewater treatment plants in Sweden (56). Analytes were extracted from raw sewage water, final effluent, and sludge. Analysis of weekly mass flows showed that many were partly eliminated during wastewater treatment, and the highest amounts were found in the sludge. Sulfamethoxazole and trimethoprim were the only analytes that showed relatively equal mass flows between the raw sewage and the final effluent, suggesting that these antibiotics are only removed to a very minor extent. Nakata et al. used LC/ESI-MS, along with LC/fluorescence detection to measure fluoroquinolone antibiotics in wastewaters effluents and surface waters in the United States and Canada (57). Ofloxacin was detected in secondary and final effluents of a wastewater plant in Michigan at concentrations of 204 and 100 ng/L, respectively, which indicated incomplete removal at the wastewater treatment plant. A mass flow calculation revealed a discharge rate of 4.8 g/day of this pharmaceutical.

Sludge was the focus of several other studies. In one of the more extensive studies, Ternes et al. used LC/ESI-MS/MS and GC/MS to measure several pharmaceuticals (antiphlogistics, lipid regulators, the antiepileptic carbamazepine, cytostatic agents, and the psychiatric drug diazepam) as well as iodinated contrast media and two musk fragrance compounds in activated and digested sludge (58). Ultrasonic solvent extraction with methanol/acetone or pressurized liquid extraction with 100% methanol was used to extract these compounds from sludge. Further cleanup was performed using C_{18ec} material and silica gel media. These methods allowed for quantification as low as 20 or 50 ng/g in activated and digested sludge, and the antiphlogistic diclofenac was found at levels ranging from 0.20 to 0.45 μg/g in five samples taken from three different municipal sewage treatment plants in Germany. Due to differences observed in sludge matrixes (particularly where there are unique compositions from industrial inputs), it was recommended to determine individual recoveries in each matrix to avoid underdeterminations of the analytes. Pressurized liquid extraction was also used in another study by Göbel et al. to extract sulfonamide and macrolide antimicrobials and trimethoprim from sewage sludge (59). LC/ESI-MS/MS was used for detection. Of the antimicrobials investigated, sulfapyridin, sulfamethoxazole, trimethoprim, azithromycin, clarithromycin, and roxithromycin were detected in municipal sewage sludge samples from Germany and Switzerland. Concentrations ranged up to 197 μg/kg (dry weight) and were generally higher in samples from Germany.

Pharmaceuticals in soil irrigated with reclaimed water was the focus of another interesting study by the USGS (60). This study represents the first of its kind for analyzing pharmaceuticals in reclaimed water used for irrigation. In this study, Kinney et al. used LC/ESI-MS to measure 19 pharmaceuticals from soil cores collected from three sites in Colorado, where reclaimed water (derived from urban wastewater) was used for irrigation. The sites chosen were from a medium-sized city in Colorado, which included landscaping in front of the city hall, a golf course, and landscaping in front of the city's reclaimed water facility. Accelerated solvent extraction was used to extract the pharmaceuticals from the soils. The majority of the pharmaceuticals measured were found in most of the samples, with erythromycin, carbamazepine, fluoxetine, and diphenhydramine being the most common. Levels were generally low (0.02−15 μg/kg of dry soil), but several pharmaceuticals were found at increased levels in the soil, indicating that some accumulate. Also, there was evidence that some pharmaceuticals may be sufficiently mobile to leach through the top 30 cm of the soil and potentially into the deeper soil layers.

While the vast majority of methods developed for pharmaceuticals involve the use of LC/MS(MS), there are a few methods that have been developed using GC/MS and CE−MS. Because CE−MS methods developed to-date are generally in the high-microgram per liter detection limit range, their utility for environmental samples for nanogram to low microgram per liter measurements has not yet been realized. However, GC/MS methods have been developed that allow comparable sensitivity to some LC/MS methods. For example, Lin et al. developed a particularly clever on-line derivatization-GC/MS method to measure clofibric acid, ibuprofen, carbamazepine, naproxen, ketoprofen, and diclofenac in water samples (61). Oasis HLB cartridges

were used for extraction of the analytes. The derivatization was accomplished using tetrabutylammonium salts that were coinjected with a large-volume injection (10 μL) of the water extract. Resulting butylated derivatives were then measured using electron ionization (EI)-MS. This derivatization technique provided sensitive, fast, and reproducible results; quantification limits ranged from 1.0 to 8.0 ng/L. Lamas et al. developed a SPME-GC/MS method for measuring serotonin reuptake inhibitors (venlafaxine, fluvoxamine, fluoxetine, citalopram, and sertraline) in environmental waters and wastewater (62). To aid in the extraction of some of the analytes, in situ acetylation was used prior to extraction. Detection limits were at the submicrogram per liter level, and no matrix effects were observed in wastewater samples.

Several interesting studies have recently focused on the oxidation, microbial degradation, and photodegradation of pharmaceuticals. For example, ozonation has been investigated as a means to oxidatively remove pharmaceuticals in wastewater and drinking water treatment. Huber et al. carried out a pilot study to determine how effectively ozonation could remove pharmaceuticals from wastewater (63). LC/ESI-MS/MS was used to measure the analytes. Results showed 90−99% removal (by oxidation) of macrolide and sulfonamide antibiotics, estrogens, and acidic pharmaceuticals (diclofenac, naproxen, indomethacin). In contrast, X-ray contrast media and some acidic pharmaceuticals were only partially oxidized. These results show that many pharmaceuticals can be efficiently oxidized by ozone and that suspended solids in wastewater treatment had only a minor influence on this process. In another study by Huber et al., the oxidation (by ozone) was investigated for the oral contraceptive 17α-ethinylestradiol (EE2) (64). Oxidation products were identified using LC/ESI-MS/MS and GC/MS, and oxidation products of the natural steroid hormones 17β-estradiol (E2) and estrone (E1) were also identified. Ozonation at doses typically applied for drinking water treatment was found to oxidize these compounds and to reduce the estrogenic activity significantly, making it a promising tool for control of these compounds in drinking water (and wastewater).

Reactions of chlorine with fluoroquinolone antibacterial agents was the focus of another study by Dodd et al. (65). This study, which used LC/ESI-MS and GC/MS to measure reaction products, elucidated the kinetics of the reactions of ciprofloxacin and enrofloxacin with chlorine. They concluded that these reactions may not completely remove the original antibacterial activity. Zühlke et al. used an in situ derivatization-GC/MS method to identify two new microbial metabolites of phenazone-type drugs (66). These metabolites [1,5-dimethyl-1,2-dehydro-2-pyrazolone and 4-(2-methylethyl)-1,5-dimethyl-1,2-dehydro-3-pyrazolone] were found at microgram per liter levels in source waters and in finished drinking water.

Three studies addressed the photodegradation of pharmaceuticals. Lin and Reinhard irradiated water samples containing gemfibrozil, ibuprofen, ketoprofen, naproxen, and propanolol and four estrogens, and used LC/ESI-MS/MS and LC/APPI-MS to follow the degradation (67). In river water, photodegradation rates were relatively fast, with half-lives ranging from 1 min to 4 h. Half-lives were longer for the estrogens measured. Ferrer et al. used LC/ESI-TOF-MS and GC/MS to investigate the photodegradation products of triclosan in wastewater (68) and in environmental waters under natural sunlight conditions (69). In the wastewater

investigation, four new photodegradation products were identified. Exact mass determinations from TOF-MS were important for their identification. The major degradation pathways involved the replacement of chlorine atoms by hydroxyl groups and chlorine losses (68). In the freshwater photolysis study, 13 phototransformation products were identified (69). GC/MS was used to identify six of them, and LC/ESI-TOF-MS was used to identify seven polar transformation products. The main photoproduct was identified as 8-chloro-9H-carbazole-1-yl-acetic acid.

Endocrine Disrupting Compounds and Hormones. Certain synthetic and natural chemicals have the ability to mimic hormones and, thus, are able to interfere or disrupt normal hormonal functions. EDCs are of high concern due to their ecotoxicological and toxicological potencies. A variety of natural compounds and anthropogenic chemicals are known or predicted to influence the endocrine system, such as natural estrogens (e.g., 17β-sitosterol, estrone), natural androgens (e.g., testosterone), phytosteroids (e.g., 17β-sitosterol), isoflavenoids (e.g., daidzeine), synthetic estrogens (e.g., 17β-ethinylestradiol), pesticides (e.g., atrazine), phthalates, alkylphenol ethoxylate surfactants, dioxins, coplanar PCBs, parabenes (hydroxybenzoate derivatives), bisphenol A, and organotins (2). Due to the enormous number of chemicals with different modes of action and different affinities to hormonal receptors (e.g., estrogen, androgen, thyroid, AH receptor), their EDC potencies differ substantially. In wildlife, EDCs are suspected of being responsible for the decline in certain species (e.g., possible increased sterility in the American alligator), change of sex in fish and shellfish, and other problems. EDCs are also suspected in declining sperm counts in humans, although this has not been proven. Both natural estrogens and synthetic EDCs can reach the aquatic environment through wastewater discharges. Fish and wildlife can be exposed, and humans can become exposed through the intake of this water into drinking water treatment plants. Many efforts have been undertaken for the accurate analysis of estrogens in aqueous and solid samples down to nanogram per liter, and even subnanogram per liter, concentrations. The first methods at the end of the 1990s used primarily GC/MS or GC/ion trap-MS/MS detection. Today, an increasing number of methods use LC/MS and LC/MS/MS. The main benefits of LC/MS/MS, in comparison to GC/MS, are lower statistical errors and no need for derivatization (2). However, when resolution is mandatory to separate isomers or congeners (such as for PCBs, dioxins, or brominated flame retardants), GC/MS/MS systems are still the method of choice.

In a recent review article, Kuster et al. discusses the analysis and distribution of estrogens and progestogens in sewage sludge, soils, and sediments (70). Sources, including treated wastewater, untreated discharges, and runoff of manure and sewage sludge used in agriculture, are discussed, as well as their fate and distribution in the environment. Physicochemical properties are also outlined, and analytical methods are summarized (including GC/MS and LC/MS methods utilizing various extraction procedures).

New LC/MS/MS methods developed include one by Reddy et al. that has extremely low detection limits of 0.04−0.25 ng/L and uses isotope dilution to overcome matrix suppression in measuring sewage influent and effluent samples (71). An automated on-line SPE-LC/ESI-MS/MS method was developed by Rodriguez-Mozaz that allows rapid and high-throughput measurements of several estrogens at 0.02−1.02 ng/L detection limits (72). Benijts et al. developed a method using SPE combined with two LC/ESI-MS/MS runs (in negative and positive ion modes) to measure 35 EDCs in environmental waters (73). Pojana et al. developed a LC/ESI-MS/MS method to measure natural and synthetic estrogens in coastal lagoon waters (74). Low method detection limits of 0.1−2.6 ng/L were achieved, and the method was applied to the analysis of lagoon waters from Venice, Italy, where low-nanogram per liter concentrations were found. Mitani et al. developed a rapid, fully automated method using in-tube SPME with LC/ESI-MS/MS to measure five estrogens in environmental waters (75). This in-tube SPME method showed significantly higher sensitivity than the direct injection method.

Several nice occurrence studies have also been published using LC/MS methods for analysis. In two such studies, the analysis of estrogens in 20 wastewater treatment plants in Japan (76) and in surface waters from the Baltic Sea (77) was conducted. In the wastewater study using LC/ESI-MS/MS, median concentrations of estrogens ranged from nondetect to as high as >100 ng/L (76). In the Baltic Sea study using LC/ESI-MS/MS, estrogens were found at levels of 0.10−17 ng/L (77).

Several new GC/MS methods have also been developed for measuring hormones and EDCs in the last two years. Noppe et al. developed a GC/MS/MS method to measure four environmental estrogens in estuarine waters at concentrations below 1 ng/L (78). This method was then applied to polluted estuary water samples from Belgium and The Netherlands. In this study, E1 was detected most frequently at concentrations up to 7 ng/L. Kawaguchi et al. developed a method using stir bar sorptive extraction, in situ derivatization, and thermal desorption-GC/MS for measuring phenolic xenoestrogens (2,4-dichlorophenol, 4-tert-butylphenol, 4-tert-octylphenol, 4-nonylphenol, pentachlorophenol, bisphenol A) in water samples (79). Detection limits ranged from 0.5 to 5 ng/L, and recoveries were good−generally higher than 94%. Yang et al. developed a SPME method with on-fiber silylation and GC/MS detection for measuring octylphenol, nonylphenol, diethylstilbestrol, dehydroisoandrosterone, E1, E2, testosterone, and pregnenolone in environmental and biological samples (80). Detection limits were in the low- to high-nanogram per liter range, and this method was used to measure these EDCs in fish blood serum and river water. Liu used microwave-assisted extraction followed by GC/MS to measure EDCs in river sediments (81). This method allowed the measurement of estrogens, 4-nonylphenol, 4-tert-octylphenol, and bisphenol A in river sediments collected in the United Kingdom. Finally, Wang et al. used SPE with GC/MS to measure estrogens and 4-tert-octylphenol, 4-nonylphenol, bisphenol A, di-n-butyl phthalate, diisobutyl phthalate, and diethylhexyl phthalate in water samples from a wastewater plant in China (82). The average removal efficiencies varied from 30 to 82% and showed that EDCs were not completely removed during wastewater treatment.

SUNSCREENS/UV FILTERS

The analysis of sunscreens/organic UV filters in water has increased substantially the last 2−3 years, so this new category of emerging contaminant is included in this review for the first

time. There are two types of UV filters—organic UV filters, which work by absorbing UV light, and inorganic UV filters (TiO_2, ZnO), which work by reflecting and scattering UV light. Organic UV filters are increasingly used in personal care products, such as sunscreens, and in cosmetics, beauty creams, skin lotions, lipsticks, hair sprays, hair dyes, and shampoos (83). Examples include benzophenone-3 (BP-3), octyl dimethyl-*p*-aminobenzoic acid, 4-methylbenzylidene camphor (4-MBC), ethylhexyl methoxycinnamate (EHMC), octyl methoxycinnamate (OMC), octocrylene (OC), butyl methoxydibenzoylmethane (BM-DBM), terephthalylidine dicamphor sulfonic acid, ethylhexyl triazone, phenylbenzimidazolesulfonic acid, ethylhexyl salicylate, and 1-(4-*tert*-butylphenyl)-3-(4-methoxyphenyl)-1,3-propanedione (BPMP). The majority of these are lipophilic compounds (low water solubility) with conjugated aromatic systems that absorb UV light in the wavelength range of 280−315 (UVB) or 315−400 nm (UVA) (84). Most sunscreen products contain several UV filters, often in combination with inorganic micropigments (84).

Because of their use in a wide variety of personal care products, these compounds can enter the aquatic environment indirectly from showering, washing off, washing clothes, etc., via wastewater treatment plants and also directly from recreational activities, such as swimming and sunbathing in lakes and rivers. There is concern over these compounds because endocrine and developmental toxicity effects have recently been reported (85). In an in vivo toxicity study involving rats, the estrogenic UV filter, 4-MBC was found to affect the development of male and female rat pups, resulting in adverse effects in the central nervous system, reproductive organs, and brain regions. 3-Benzylidene camphor also exhibited development toxicity, with different effect patterns. Levels of 4-MBC recently observed in Swiss lakes are close to the doses that caused these effects in the animals. Both reproductive organs and the central nervous system appeared to be the most sensitive targets to developmental effects of these compounds.

Recent studies of UV filters include their measurement in lakes, rivers, and the influents and effluents of wastewater treatment plants. There have also been investigations of aqueous photolysis reactions and halogenation reactions of UV filters in swimming pool water. GC/MS has been primarily used to track the organic UV filters and identify reaction products, since the majority of these UV filter compounds are lipophilic. Also, because they are lipophilic, extreme care must be taken during sampling and sample preparation, as these UV filter compounds are easily transferred to glassware and consumables, which can contribute to analytical blank problems (84). Poiger et al. carried out an occurrence study of nine organic UV filter compounds from sunscreens in two Swiss lakes where recreational swimming is popular (84). SPE with GC/MS allowed low-nanogram per liter detection limits; semipermeable membrane devices (SPMDs) were also used for passive sample collection and to determine the potential for bioaccumulation. Concentrations measured (<2−125 ng/L) were lower than predicted based on surveys taken from swimmers and bathers at these lakes. This was proposed to be due to (1) an overestimation of these inputs (e.g., less than the 50% wash-off of UV filters assumed to occur during swimming), and (2) some removal of these compounds from the lakes by

degradation or sorption/sedimentation. UV filters were detected in the SPMDs at concentrations of 80−950 ng per SPMD, which indicated a potential for bioaccumulation.

Another nice occurrence study by Balmer et al. measured four organic UV filters in the influent and effluent of wastewater treatment plants, in surface waters from four Swiss lakes and a river, and in fish collected from six Swiss lakes (83). The maximum concentration of UV filters in wastewater influents was 19 μg/L and was much lower in the treated effluent water, indicating substantial elimination of these compounds in the treatment plants. UV filters were detected in all surface waters sampled, but were all at nanogram per liter levels, with a maximum of 125 ng/L observed for BP-3 in one of the lakes. 4-MBC was the most prevalent compound measured, followed by BP-3, EHMC, and OC. No UV filters were detected in the remote mountain lake sampled. All fish analyzed contained low concentrations of UV filters, with a maximum of 5 (whole fish) and 166 ng/g lipid.

Giokas et al. developed an analytical method using SPE with both LC-diode array detection and GC/MS to quantify four UV filters (BP-3, 4-MBC, BPMP, OMC) in natural waters (86). The method was used to measure these UV filters in coastal waters from northwestern Greece and in shower wastewater. Sunscreen residues were found up to 10 ng/L. Giokas et al. developed a different extraction procedure in a subsequent paper to improve recoveries of UV filters from coastal waters. This method used surfactant-mediated extraction to improve recoveries and GC/MS detection (87). Recoveries ranged from 95 to 102%.

DRINKING WATER DISINFECTION BYPRODUCTS

In addition to new regulations involving DBPs (e.g., the Stage 2 Disinfectants/DBP Rule), there are also new, emerging issues with DBPs (88). New human exposure research is revealing that ingestion is not the only important route of exposure—inhalation from showering and dermal absorption (from bathing and other activities) can provide equivalent exposures or increased exposures to certain DBPs (88). Therefore, these exposure routes are now being recognized in new epidemiologic studies that are being conducted. And epidemiology studies are beginning to focus more on reproductive and developmental effects—which recent studies have shown to be important.

Toxicologically Important DBPs. DBPs beyond those that are currently regulated are becoming important. For example, brominated DBPs are now being recognized as toxicologically important because brominated DBPs are proving to be much more carcinogenic than their chlorinated analogues (88), and preliminary studies are indicating that iodinated compounds may be more toxic than their brominated analogues (89). Brominated and iodinated DBPs form due to the reaction of the disinfectant (such as chlorine) with natural bromide or iodide present in source waters. Coastal cities, whose groundwaters and surface waters can be impacted by saltwater intrusion, and some inland locations, whose surface waters can be impacted by natural salt deposits from ancient seas or oil field brines, are examples of locations that can have high bromide and iodide levels. A significant proportion of the U.S. population and several other countries now live in coastal regions that are impacted by bromide and iodide;

therefore, exposures to brominated and iodinated DBPs are important. Early evidence in epidemiologic studies also gives indication that brominated DBPs may be associated with the new reproductive and developmental effects, as well as cancer effects.

Specific DBPs that are of current interest include iodo acids, bromonitromethanes, iodotrihalomethanes (THMs), brominated forms of MX (MX is 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone), and nitrosodimethylamine (NDMA; which is not brominated, but is classified as a probable carcinogen) (88). Iodoacetic acid, one of five iodo acids identified for the first time in chloraminated drinking water, has recently been shown to be more genotoxic and cytotoxic to mammalian cells than all DBPs that have been studied, including the regulated haloacetic acids (HAAs) and bromate (89). It is a factor of $2\times$ more genotoxic than bromoacetic acid, which is the most genotoxic of the regulated HAAs. Other iodo acids identified—bromoiodoacetic acid, (Z)-3-bromo-3-iodopropenoic acid, (E)-3-bromo-3-iodopropenoic acid, and (E)-2-iodo-3-methylbutenedioic acid (89)—have been synthesized and are currently under investigation for genotoxic and cytotoxic effects. They were initially discovered in chloraminated drinking water extracts using methylation with GC/high-resolution-EI-MS. Analytical methods for the five iodo acids are currently under development for an occurrence study to determine their concentrations in chloraminated drinking water. These iodo acids are not only of concern for their potential health risks, but also because early research indicates that they may be maximized (along with iodo-THMs) in waters treated with chloramines. Chloramination has become a popular alternative to chlorination for plants that have difficulty meeting the regulations with chlorine, and its use is expected to increase with the advent of the new Stage 2 D/DBP Rule. Chloramines are generated from the reaction of chlorine with ammonia, and it appears that the length of free chlorine contact time (before ammonia addition to form chloramines) is an important factor in the formation of iodo acids and iodo-THMs. Because of chlorine's competing reaction to form iodate as a sink for the natural iodide, it is likely that plants with significant free chlorine contact time before the addition of ammonia will not produce substantial levels of iodo acids or iodo-THMs.

The bromonitromethanes (including dibromonitromethane, tribromonitromethane, and bromonitromethane) have been recently shown to be extremely cytotoxic and genotoxic to mammalian cells (88). For example, dibromonitromethane is at least 1 order of magnitude more genotoxic to mammalian cells than MX and is more genotoxic than all of the regulated DBPs, except for monobromoacetic acid. Bromonitromethanes have been found to be DBPs formed by chlorination or chloramination and have been shown to be increased in formation when preozonation is used before chlorine or chloramine treatment. Bromonitromethanes, iodo-THMs, brominated forms of MX (so-called BMXs), as well as other "high-priority" DBPs were the focus of a U.S. Nationwide DBP Occurrence Study (88). This Nationwide Occurrence Study focused on ~50 high-priority DBPs that were selected from an extensive prioritization effort (according to predicted cancer effects) of all DBPs that have been reported.

A recent review article summarizes these new DBP issues, as well as different routes of exposure to them (88). Another review by Zwiener and Richardson discusses the use of LC/MS for identifying and measuring highly polar DBPs and high molecular weight DBPs in drinking water (90). Useful derivatization techniques, as well as related MS techniques, such as FAIMS-ESI-MS, ion chromatography (IC)/ESI-MS, and membrane introduction MS, are also discussed. This review covers not only traditional DBPs that are formed by the reaction of the disinfectant (oxidant) with natural organic matter but also covers newly identified DBPs that are formed by the reaction of the disinfectant with contaminants. Examples of those include reaction products with estrogens, alkylphenol ethoxylates, pesticides, and algal toxins.

Combined Toxicity—Analytical Chemistry Approaches. Traditionally, analytical chemistry studies of DBPs (identification and occurrence) have been carried out separately from toxicity studies of DBPs. There is new work underway in combining toxicity testing and chemical identification to aid in the discovery of toxicologically important DBPs and in understanding the toxicity of the drinking water mixture or the new DBPs identified. This was done in the study cited earlier for the identification of the iodo acid DBPs (89), and it was also done in a study by Monarca et al. that investigated the chemical DBP composition and toxicity of drinking water treated with a new disinfectant, peracetic acid (91). In that study, GC/MS was used to comprehensively identify the DBPs formed. This approach was also used in a paper published by Gong et al., where a new DBP was identified, isolated, and tested for mutagenicity (92). The new DBP was identified as 2,2,4-trichloro-5-methoxycyclopent-4-ene-1,3-dione, through the combined use of Fourier transform (FT)-infrared (IR) spectroscopy, EI-MS, ^{1}H and ^{13}C NMR spectroscopy, and single-crystal X-ray diffraction (92). Ames test results showed it to be highly mutagenic.

Addressing Highly Polar DBPs. Because many of the unidentified DBPs are believed to be highly polar, efforts are being made to uncover them. One approach is to use derivatization with GC/EI-MS, as was done previously to discover highly polar carbonyl DBPs in drinking water. Along these lines, Vincenti et al. developed fluorinated chloroformate derivatizing reagents that can derivatize highly polar compounds containing alcohol, amine, and carboxylic acid functional groups (93). This derivatization is done in situ in the water using ultrasonication, and it renders the highly polar polyalcohols, amines, and acids nonpolar, which allows them to be extracted and analyzed by GC/electron capture negative ionization (ECNI, also called negative chemical ionization)-MS. The entire procedure, from raw aqueous sample to ready-to-inject hexane solutions of derivatives takes less than 10 min. Of the four reagents investigated, 2,2,3,3,4,4,5,5-octafluoro-1-pentyl chloroformate showed the best performance overall, with good reaction efficiency, good chromatographic and spectroscopic properties, low detection limits (10—100 fmol), and a linear response of more than 2 orders of magnitude. Another reagent, 2,3,4,5,6-pentafluorobenzyl chloroformate, showed ideal applicability for derivatizing amino alcohols and amino acids. This method was subsequently used to identify DBPs in simulated ozonated drinking water (aqueous reaction of fulvic acid with ozone), and three highly polar DBPs were discovered.

A new LC/MS method was developed by Dixon et al. to quantify dichloroacetic acid (which is highly polar) without the

traditional derivatization that is used to measure HAAs by GC/MS (94). This method uses HILIC−LC/MS/MS and can quantify dichloroacetic acid in drinking water without derivatization. Common methods used to measure HAAs in drinking water generally involve the use of methylating agents (e.g., diazomethane, H_2SO_4/methanol, BF_3/methanol), two of these involving the use of strong acids. This HILIC−LC/MS/MS method was developed because of a recent finding that trichloroacetic acid can convert to dichloroacetic acid with acid methylation, which would result in artificially high levels of dichloroacetic acid being reported. HILIC is a method by which the aqueous solvent, rather than the organic solvent, determines how quickly the compound elutes. HILIC columns contain a polar end group (such as an amino group), and retention is based on the affinity of the polar analyte for the charged end group of the column stationary phase. The use of HILIC allowed dichloroacetic acid to elute away from the solvent front. Other methods have used ion-pairing agents to separate HAAs by LC, but the use of ion-pairing reagents can suppress ionization in the mass spectrometer. With this HILIC−LC/MS/MS method, detection limits of 5 ng/mL (μg/L) were achieved using a 500-μL water sample. This method is the only method for dichloroacetic acid analysis that has been validated using the criteria recommended by the U.S. Food and Drug Administration (FDA).

Other DBP Studies Using GC/MS. In other DBP studies using traditional GC/MS (which is still the most common technique used for DBPs), Huang et al. investigated the formation of DBPs from the ozonation of polluted surface waters (95). DBPs identified include carboxylic acids, benzoic compounds, aliphatic and odorous aldehydes, bromoform, bromoacetic acid, dibromoacetic acid, 2,4-bromophenol, and dibromoacetonitrile. The type of natural organic matter (NOM) was also found to affect the proportions of DBPs produced. For example, humic acid showed the highest bromoform, dibromoacetic acid, and 2,4-dibromophenol levels, whereas hydrophilic neutral NOM produced less bromoform and 2,4-dibromophenol, but the highest levels of dibromoacetonitrile. In addition GC−high-resolution-EI-MS was used to identify 59 different organic compounds in the drinking water, including aromatics, acids/esters, alcohols, aldehydes, phthalates, and amines/amino acids. The effect of humic substances on DBP formation was also investigated by Nikolaou et al. (96). GC/MS was used to investigate the formation of 24 DBPs from several different DBP classes (96). The content of the humic substances was found to influence the formation of DBPs, especially THMs, trichloroacetic acid, dibromoacetic acid, chloral hydrate, 1,1-dichloropropanone, and 1,1,1-trichloropropanone. In contrast, the formation of dichloroacetic acid did not seem to be affected by the humic substances content.

As with other types of emerging contaminants, issues with reclaimed water have also become important in considering DBPs. To that end, Nurizzo et al. used GC/MS to investigate DBPs formed when biologically treated and filtered wastewater is disinfected with sodium hypochlorite (one form of chlorine), peracetic acid, or UV irradiation (97). When hypochlorite was used, the THMs exceeded regulations, whereas no significant DBPs were observed when this water was treated with peracetic acid or UV.

In one the more extensive occurrence studies conducted the last two years, Charrois et al. used GC/MS to measure THMs, HAAs, and total organic carbon (TOC) in 11 rural Alberta, Canada, communities (98). These DBPs were also followed through the distribution system, as well as at the treatment plant, to determine if levels increased or decreased in the distribution system. THM levels (chloroform, bromodichloromethane, chlorodibromomethane) were found to exceed Canada's guidelines, often exceeding 100 μg/L, and source waters with the highest TOC concentrations (15 mg/L) showed the highest THM levels (200 μg/L).

While GC/MS, and sometimes LC/MS, are used to characterize DBPs, there have also been a few methods developed using CE−MS. In one such method, SPME and CE/ICPMS were used to measure iodophenols (2-iodophenol, 4-iodophenol, 2,4,6-triiodophenol) in water (99). Halophenols can be found as pollutants, but have also been found to be drinking water DBPs. Detection limits were at the submicrogram per liter level.

High Molecular Weight DBPs. New work continues to address high molecular weight DBPs (>500 Da), which have been shown to be a major part of the unknown fraction of DBPs. ESI-MS/MS and MALDI-MS are allowing researchers to study this more in depth. Most of this work is very preliminary, due to the complexity of the mass spectra obtained ("a peak at every mass" situation). Minear's group from the University of Illinois has been one of the groups leading this area of research. In a 2005 study, Minear and collaborators investigated high molecular weight DBP material from chlorinated Suwannee River fulvic acid with and without coagulation pretreatment (100). Fractions were collected using size exclusion chromatography, and the high molecular weight fractions were analyzed by negative ion-ESI-MS and ESI-MS/MS. Each fraction showed a distribution of ions from m/z 10 to 4000, with most ions present in the m/z 100−500 region. For the high molecular weight fractions, the ion intensities in fractions with coagulation pretreatment were much weaker than those in the corresponding fraction without coagulation pretreatment, indicating that coagulation may be significantly reducing the formation of high molecular weight DBPs. Precursor (parent) ion scans of m/z 35 (chlorine) were found to be useful for uncovering chlorine-containing DBPs, and product (daughter) ion scans were performed to confirm the presence of chlorine. However, full scan product ion spectra were too complex to allow definitive structural information. It was suggested that it is unlikely that any particular MS peak in the ESI-MS spectrum is due solely to a single structural or compositional isomer. However, this work does demonstrate that high molecular weight chlorine-containing DBPs are formed during chlorination, and it is an advance in the area of high molecular weight DBPs. In a related study from Minear's group, radiolabeled chlorine (^{36}Cl) was used to further probe high molecular weight DBPs formed upon chlorination of drinking water (101). Results of this study showed that the amount of high molecular weight DBPs decreased with the increase in chlorine contact time, but increased with increasing pH 9 (from 5.5 to 9.5), and was less in ultrafiltered samples from fulvic acid than humic acid.

NDMA and Other Nitrosamines. Until recently, concerns about NDMA primarily stemmed from its presence in food, beverages, consumer products, contaminated groundwater

(from the use of rocket fuel), and polluted air (e.g., tobacco smoke). However, it has recently become evident that NDMA is also a drinking water DBP, which could make human exposure more widespread. It has primarily been found in chloraminated drinking water, where the nitrogen in monochloramine (NH$_2$Cl) is incorporated into the structure of the NDMA byproduct formed. Chlorination can also form NDMA to some extent, when there are nitrogen precursors present (e.g., natural ammonia in the source water or nitrogen-containing coagulants used in the water treatment process). NDMA was initially discovered in chlorinated drinking waters from Ontario, Canada, and has recently been found in other locations and in laboratory studies. The observation of NDMA in U.S. waters is largely due to improved analytical techniques that have allowed its determination at low nanogram per liter concentrations. Recent measurements have shown it is generally present at low nanogram per liter in chloraminated/chlorinated drinking water, but it can be formed at much higher levels in wastewater treated with chlorine. While NDMA and other nitrosamines are not currently regulated in the United States, they are now on the U.S. EPA's newest Unregulated Contaminants Monitoring Rule (UCMR-2), and nationwide occurrence data will be collected for future regulatory determination (www.epa.gov/safewater/ucmr). In addition, California has set an action level of 0.01 μg/L for NDMA in groundwater/drinking water. Canada (as a country) does not regulate NDMA, but Ontario has issued an interim maximum acceptable concentration for NDMA at 9 ng/L (www.ene.gov.on.ca/envision/gp/4449e.pdf).

Andrzejewski et al. published a nice review on NDMA in 2005, where its toxicological issues, mechanisms of formation in drinking water treatment, and physiochemical properties are discussed (102). This review also mentions the possibility of forming NDMA with chlorine dioxide disinfection (via reactions with dimethylamine).

Determination of NDMA in water can be very difficult because it is generally present at low nanogram per liter levels, and it has a low octanol–water partition coefficient (102). In addition, MS measurement is somewhat limited because NDMA has a relatively low molecular weight. As a result, only two ions of NDMA can be utilized with EI-MS (m/z 42 and the molecular ion m/z 74, which is also an ion that is common to many other compounds). NDMA is also relatively volatile and has a short GC retention time, which allows it to potentially coelute with many other low molecular weight substances. A new EPA method, which uses MS/MS, helps to overcome these issues. This method was created for measuring NDMA and six additional nitrosamines in drinking water (EPA Method 521, Determination of Nitrosamines in Drinking Water by Solid-Phase Extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS)) (www.epa.gov/nerlcwww/m_521.pdf). This method, created in September 2004, was developed for inclusion in the UCMR to enable the collection of nationwide occurrence data on nitrosamines in drinking water for regulatory determination. If NDMA or other nitrosamines become regulated drinking water contaminants in the future, the method could also be used for compliance monitoring (www.epa.gov/nerl/research/2004/g2-6.html). This method enables the measurement of NDMA and six other nitrosamines (N-nitroso-methylethylamine, N-nitrosodiethylamine, N-nitrosodi-n-propylamine, N-nitrosodi-n-butylamine, N-nitrosopyrrolidine, N-nitrosopiperidine) in drinking water at detection limits ranging from 1.2 to 2.1 ng/L. This method is an improvement over previously published methods for nitrosamines, in that the sample preparation steps are simple, efficient, and inexpensive; the use of tandem-MS provides positive identification of all analytes without the use of additional confirmatory methods; and quality control steps ensure precision and accuracy.

Probably the most significant study of NDMA and nitrosamines in the last two years was the report of the highest levels to-date of NDMA in real finished drinking waters (180 ng/L) and the discovery of nitrosamines beyond NDMA in finished drinking water. Charrois et al. discovered two new nitrosamines—N-nitrosopyrrolidine and N-nitrosomorpholine—in finished drinking water (both at the plant and in the distribution system) from two cities in Canada that use chloramination for treatment (103). This represents the first report of other nitrosamines besides NDMA in drinking water. Levels of N-nitrosopyrrolidine ranged from 2 to 4 ng/L, and N-nitrosomorpholine was found in drinking water from one city at 1 ng/L. NDMA levels ranged from 2 to 180 ng/L. This 180 ng/L level, which was found in the distribution system of one city, is the highest to-date concentration reported for NDMA in drinking water. The data in this study indicate that NDMA (and other nitrosamines) can continue to form in the distribution system and show dramatically increased levels in the distribution system as compared to the drinking water treatment plant (e.g., from an initial 67 ng/L NDMA at the plant to 180 ng/L in the distribution system). This suggests that previous measurements of NDMA at the treatment plant may substantially underestimate the public's exposure to this probable carcinogen. A SPE-GC/positive ion-CI-MS method (with 0.4–1.6 ng/L detection limits), which used both isotope dilution, surrogate standards, and internal standards, was used to measure these nitrosamines.

DBPs of Pollutants. As mentioned previously, DBPs are going beyond the "classic" DBPs formed by the reaction of NOM with disinfectants, such that reactions of pollutant material with disinfectants are now being studied. The last two years have produced studies of disinfectant reactions with pharmaceuticals, antibacterial agents, nonylphenol ethoxylate surfactants, and algal toxins. The oral contraceptive EE2 was the focus of a study using ozonation (104) and chlorination (105). Ozonation doses commonly used in drinking water treatment were shown to be sufficient to reduce the estrogenicity by a factor of >200, and LC/ESI-MS/MS and GC/EI-MS were used to identify the reaction products formed. The chemical structures of the oxidation products were significantly altered compared to the parent compounds, which explained the diminished estrogenic activity after ozonation. Moriyama et al. used preparative LC, MS, and NMR to identify reaction products of EE2 with chlorine (105). Six products were formed, and the two major products were identified as 4-chloroethinylestradiol (2-ClEE2) and 2,4-dichloro-ethinylestradiol (2,4-diClEE2). 2-ClEE2 showed estrogenic activity similar to that of the parent EE2 compound, but 2,4-diClEE2 had ~10 times lower estrogenic activity. Oxidation products from the reaction of pharmaceuticals with chlorine dioxide treatment were the focus of another study by Huber et al. (106). LC/ESI-MS/MS was used to identify the products. Out of the nine pharma-

ceuticals investigated, only four showed appreciable reactivity with chlorine dioxide: the sulfonamide antibiotic sulfamethoxazole, the macrolide antibiotic roxithromycin, the estrogen EE2, and the antiphlogistic diclofenac. Results showed that chlorine dioxide also reacted rapidly with other sulfonamides and macrolides, the natural hormones E1 and E2, as well as three pyrazolone derivatives.

Dodd and Huang used LC/ESI-MS, along with fraction collection and GC/MS and ¹H NMR, to investigate the reaction chlorine with the antibacterial agent, sulfamethoxazole (107). Reactions with chlorine were quite rapid (half-life of 23 s), and reaction mechanisms were elucidated. 3-Amino-5-methylisoxazole, sulfate, and N-chloro-p-benzoquinoneimine were the reaction products identified. In another study from the same group (also covered in the section on pharmaceuticals), the reactions of chlorine with fluoroquinolone antibacterial agents were also investigated (65). This study also used LC/ESI-MS and GC/MS to determine the reaction products.

Petrovic et al. used LC/ESI-MS/MS to study the ozonation of nonylphenol ethoxylate surfactants (108). Ozone was found to decompose all of the nonylphenolic compounds, including the metabolite nonylphenol, to levels below detection. Finally, Kull et al. used LC/MS/MS to investigate the oxidation of the cyanobacterial toxin, microcystin-LR by chlorine dioxide (109). The reaction products were found to be dihydroxy isomers of microcystin-LR and were nontoxic in a protein phosphatase inhibition assay. MS/MS helped to determine that the main point of attack of chlorine dioxide was directed at the two conjugated double bonds in the Adda residue of the microcystin.

POLYBROMINATED DIPHENYL ETHERS AND NEW FLAME RETARDANTS

PBDEs have been used for many years as flame retardants in a variety of commercial products including foam cushions in chairs and other furniture, plastics, textile coatings, electronic appliances, and printed circuit boards. Of the 175 different types of flame retardants, the brominated ones dominate the market due to their low cost and high performance. The use of these flame retardants is believed to have successfully reduced fire-related deaths, injuries, and property damage. However, there is recent concern regarding these emerging contaminants because of their widespread presence in the environment and in human and wildlife samples and their presence in locations far from where they were produced or used. There is also strong evidence of increasing contamination. Worldwide, more than 200 000 metric tons of brominated flame retardants are produced each year, with PBDEs accounting for 67 400 metric tons/year, with more than 50% of that being used in the United States and Canada (110). The greatest health concern for potential health effects comes from recent reports of developmental neurotoxicity in mice, but there are also concerns regarding the potential for hormonal disruption and, in some cases, cancer (110). Due to concerns about potential adverse development effects and the widespread presence of these compounds, there has been a Directive established to control emissions of these compounds in Europe. There is also legislation in some individual European countries banning production and usage of some PBDEs (111). In the United States, however, it has taken more time for these contaminants to be noticed. In 2003, California voted to ban the use of octa- and pentylbromodiphenyl

ether beginning in 2008, and the only U.S. manufacturer of PBDEs has voluntarily agreed to phase out the production of the penta- and octabrominated diphenyl ether (DPE) and will be replacing the penta-DPE with another halogenated aryl ester/phosphate compound that is not persistent, bioaccumulative, or toxic. This new flame retardant will be used in polyurethane applications, such as chair cushions, where the penta-BDE was popularly used. However, there is still the possibility that these discontinued PBDEs will be imported into the United States and used in products. While PBDEs are not currently regulated on a national scale in the United States, they are currently listed on EPA's UCMR-2 for further study and potential regulation (www.epa.gov/safewater/ucmr).

Only very recently were studies from the United States beginning to be published. However, this area has experienced tremendous growth in the last two years, as evidenced by the increasing numbers of papers published and by the number of review articles. Although the most frequently used PBDE is deca-BDE, lower brominated diphenyl ethers are more often found in environmental samples (112). In contrast to the deca-BDE, which is poorly absorbed biologically, rapidly eliminated, and not bioaccumulated, the lower molecular weight congeners (tri- to hexa-BDEs) are almost completely absorbed, slowly eliminated, and highly bioaccumulated (112). The main pathway to human exposure is believed to be through food (111).

PBDE Flame Retardants. Most previous PBDE studies have focused on the measurement of PBDEs in biological samples, including human blood, milk, and tissues, as well as marine mammals and other wildlife. New studies are trying to address the source of these PBDEs in the environment, and new methods continue to be developed. Because PBDEs are hydrophobic, GC with EI-MS and ECNI-MS can be used for their measurement. High-resolution EI-MS with isotope dilution and GC/MS/MS are also increasingly being used to measure PBDEs in complex matrixes.

Reviews published in the last two years include one by D'Silva et al., which discusses the ecological and environmental impact of the use of PBDEs, polybrominated biphenyls, and other brominated flame retardants (112). This review also summarizes the occurrence, source, fate, toxicology, exposure, analysis, and future work that is needed. Domingo reviewed human exposure to PBDEs through the diet and summarizes findings of PBDEs in fish and other marine species and various foods (113). Scrimshaw et al. reviewed analytical methods for determining PBDEs in wastewaters and sewage sludge (114). Extraction, cleanup, and quantitation by GC/MS are included. In another review article, Hites summarizes PBDE concentrations measured in several environmental media and analyzes these data in terms of relative concentrations, concentration trends, and congener profiles (115). The data show increasing levels of PBDEs in human blood, milk, and tissues, as well as in marine mammals and bird eggs. A case was made that the environment and people from North America are much more contaminated with PBDEs as compared to Europe and that these levels have doubled every 4−6 yr. However, analyses did not show patterns that could be used to attribute specific sources of contamination.

Two new studies focused on potential sources of PBDEs. One of these studies is the first to suggest the importance of

wastewater treatment plant discharges as potential sources of PBDEs to the Great Lakes (116). In this study, Samara et al. used GC/MS to measure 9 PBDEs and 14 PCBs in sediments from the Niagara River (116). All sites except one showed PBDEs in sediments, with total concentrations as high as 148 ng/g. Results showed that the highest levels of PBDEs and PCBs were found in sediments collected from areas closest to the discharge locations of municipal wastewater treatment plants and local industries, which suggests wastewater discharges are important sources of PBDEs. In another source study carried out in Switzerland, Morf et al. used GC/MS to measure PBDEs and other brominated flame retardants in waste electrical and electronic equipment at a recycling plant (117). Importantly, this study discovered that other brominated substances are beginning to be used as substitutes for PBDEs in electrical and electronic equipment, and gram per kilogram concentrations were found in fine dust fractions recovered in the off-gas purification system of the recycling plant, which reveals the potential for high emissions from recycling plants.

Several other very nice occurrence studies have been conducted, where sediments, air, water, and biota have been targeted. In a Great Lakes study, Zhu and Hites used GC/ECNI-MS to measure PBDEs in sediment cores taken from Lake Michigan and Lake Erie (118). Results showed that the concentrations of total PBDEs in these sediments have increased rapidly, doubling in 5−10 years. BDE-209 was found to be the predominant congener in both sediment cores, making up 95−99% of the total PBDE load. Levels of total PBDEs were 190 and 40 ng/cm^2, for Lake Michigan and Lake Erie, respectively, and total burdens were 110 and 10 metric tons, respectively. Another study by Hoh and Hites investigated PBDEs in the air of the East-Central United States (119). Using GC/ECNI-MS and high-volume air samplers, mean PBDE concentrations were found to be 100 pg/m^3 in Chicago, which was higher than at the other sites and has increased significantly from previous measurements in 1997−1998. Lower PBDEs (tri- through hexa-BDE) were found in both the particulate and gas phases; higher BDEs (hepta- through deca-BDEs) were found mostly in the particulate phase. Additional information suggested that deca-BDEs bound to particles can move long distances from source regions to nonsource regions.

In another study, Eljarrat et al. used selective pressurized liquid extraction and GC/ECNI-MS to investigate PBDEs in coastal sediments from Spain (120). Total PBDEs ranged from 2.7 to 134 ng/g of dry weight, and as with the Great Lakes study mentioned above, BDE-209 was the predominant congener, comprising 50−99% of the total PBDE load. The San Francisco estuary was the focus of another occurrence study by Oros et al. (121). For this study, GC/EI-MS was used to measure PBDEs in water, surface sediments, and bivalves collected from the estuary. PBDE levels ranged from 3 to 513 pg/L, with the highest concentrations found in the Lower South Bay region, which receives ~26% of the estuary's wastewater treatment plant effluents.

Many new methods have also been developed in the last two years for PBDEs. GC/ion trap methods are becoming increasingly popular, as are alternative extraction methods. Larrazabal et al. developed a GC/quadrupole ion storage-MS method to measure PBDEs in environmental samples and compared this new method to more commonly used GC/ECNI-MS methods (122). Detection

limits ranged from 62 to 621 fg, which was similar to ECNI methods, and better than EI-MS. Wang et al. also developed a GC/ion trap-MS method to determine PBDEs, and utilized tandem-MS, along with isotope dilution for quantification (123). Detection limits ranged from 0.013 to 0.25 ng/g in soil, and this method was used to measure PBDEs in soil collected from an electronic waste recycling site (123). Eljarrat et al. detailed their new selective pressurized liquid extraction-GC/ECNI-MS method for measuring deca-BDE in sediments (124). Detection limits of 2 pg were achieved. Gago-Martinez et al. used SPME as an alternative for extracting three PBDEs (tri-, tetra-, and penta-BDEs) from environmental samples (125). GC/MS was used for detection, and quantification could be performed at ppt levels.

Biological samples were the focus of several other papers. Naert et al. used GC/MS/MS to measure PBDEs and PCBs in 53 human adipose tissue samples collected from men and women in Belgium (126). Total PBDE concentrations ranged from 1.23 to 57.2 ng/g of lipid weight and were comparable to levels observed in other European countries. No age dependency was found for PBDE concentrations, whereas PCB s showed higher correlations with age. Gomara et al. also used GC/MS/MS with isotope dilution to measure 10 PBDEs in human adipose tissue, serum, and food samples (127). Focant et al. developed a two-dimensional GC/isotope dilution-TOF-MS method for measuring PBDEs, PCBs, and organochlorine pesticides in human serum and milk (128). Two-dimensional GC ensured separation of most compounds, and TOF-MS allowed deconvolution of coeluting compounds. ^{13}C-Labeled internal standards were used for quantification. Method detection limits ranged from 1 to 15 pg/uL. Sjödin et al. developed a semiautomated high-throughput extraction and cleanup method for measuring PBDEs and PCBs in human breast milk (129). This method used GC/isotope dilution-high resolution-MS for detection and had method detection limits of 0.1−1.2 ng/g.

In another human exposure study, Kalantzi et al. used GC/MS to measure PBDEs in breast milk collected from 54 women in two regions from the United Kingdom (130). Total PBDE levels ranged from 0.3 to 69 ng/g of lipid, with PBDE-47 being the most abundant congener. Differences in chemical compositions were evident in the two groups of women from different regions in the UK. Dolphins were the focus of another biological sample study of PBDEs (131). In this study, Tuerk et al. used GC/MS to measure PBDEs and PCBs in dolphins collected from stranding events in Massachusetts (131). Total PBDE concentrations were found to be the highest in juvenile white-sided dolphins (2.4 μg/g of wet mass) and lowest in adult female rough-toothed dolphins (0.51 μg/g of wet mass).

New Flame Retardants. While PBDEs are the most commonly used and commonly measured flame retardants, this year there were exciting discoveries of new flame retardants found in the environment. In a 2006 finding from Hites' group, a chlorinated flame retardant—Dechlorane Plus—that has been on the market for 40 years was discovered in environmental samples (132). The compounds identified are stereoisomers (syn and anti) of bis-(hexachlorocyclopentadieno)cyclooctane. For this discovery, the authors pursued the identification of two significant unknown chlorine-containing GC/MS peaks noticed during the course of analyzing for PBDE flame retardants in environmental samples.

The compounds were identified using GC/ECNI- and GC/EI-MS. A commercial standard of this flame retardant provided a match of the mass spectra and GC retention times. Dechlorane Plus is used for coating electrical wires and cables, in computer connectors, and in plastic roofing material, and is considered a high-production-volume chemical because more than 1 million pounds of it is manufactured every year (133). In this study by Hites et al., air concentrations reached 490 pg/m^3, which are similar to concentrations of deca-BDE, the most widely used PBDE flame retardant. Dechlorane Plus was also found in sediments from the Great Lakes (and in cores dating back to the 1970s), as well as in fish. These data suggest that these chlorinated flame retardants are persistent and bioaccumulative, and this finding makes a case that not all significant contaminants have been discovered and that researchers should continue to pursue new environmental contaminants.

Hites' group also reported the discovery of two new brominated flame retardants—TBE and PEB—in air samples from various sites in the United States (134). Matches of GC retention times and mass spectra were obtained with the authentic standards, and PEB levels up to 550 pg/m^3 were found (in air from Chicago), which was 10 times higher than the concentration of total PBDEs in this sample.

BENZOTRIAZOLES

Interest in benzotriazoles is emerging, and this class of emerging contaminant is included in this review for the first time. Benzotriazoles are complexing agents that are widely used as anticorrosives (e.g., in engine coolants or antifreezing liquids) and for silver protection in dishwashing liquids (135). The two common forms, benzotriazole and tolyltriazole, are soluble in water, are resistant to biodegradation, and are not eliminated in wastewater treatment (135, 136). Because of their water solubility, LC/MS and LC/MS/MS methods have been recently developed for their measurement in environmental waters. Giger et al. developed a LC/MS/MS method, using Oasis HLB SPE cartridges for extraction and concentration (135). Using this method, benzotriazole and tolyltriazole were found at levels up to 18 and 5 μg/L, respectively, in municipal wastewater effluents. Concentrations in biologically treated effluents ranged from 8 to 12 and 2 to 3 μg/L, respectively. Weiss and Reemtsma reported another LC/ESI-MS/MS method for measuring benzotriazole and two isomers of tolyltriazole (5- and 4-tolyltriazole) in environmental waters (136). Using SPE for extraction, their method could achieve detection limits of 10 ng/L (for groundwater) and 25 μg/L (for untreated wastewater). Microgram per liter levels were found in municipal wastewater, and removal in wastewater treatment was poor, which allowed these compounds to be cycled back to surface water and to drinking water source waters. Of the two tolyltriazole isomers, the 4-tolyltriazole isomer was more stable in the environment.

NAPHTHENIC ACIDS

Naphthenic acids are becoming important emerging environmental contaminants, so they are also being included in this review for the first time. Current research is focusing on naphthenic acids that are found in oil sands region in Alberta, Canada, one of the highest producers of crude oil in the world. However, oil sands exist in other regions of the world, and as such, the naphthenic acid problem will not likely be isolated only to Alberta. In the extraction of crude oil from oil sands, caustic hot water extraction is used, which separates the bitumen from the sand, along with vast quantities of tailing water, which contains clay, sand, and organic compounds of high polarity and molecular weight (137). The tailing water is known to be toxic, and the primary toxic components have been identified as oil sand naphthenic acids (a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids that dissolve in water at neutral or alkaline pH and have surfactantlike properties). Naphthenic acids are toxic to aquatic organisms, including phytoplankton, daphnia, fish, and mammals, and are also endocrine disrupting (137). High levels of naphthenic acids are released in the extraction process, with 80−120 mg/L levels common and 0.1−0.2 m^3 of tailings per ton of oil sands processed (138). The total volume of tailing ponds is projected to exceed 10^9 m^3 by the year 2020 (138).

Naphthenic acids are challenging to measure because they are present as a complex mixture of isomers and homologues. Two-dimensional (2D)-GC and TOF-MS are currently enabling researchers to better separate the complex mixture of naphthenic acids (through greater chromatographic peak capacity and fast scanning of the TOF-MS instrument) and to identify the individual compounds (through the use of exact mass data provided by TOF-MS). To this end, Hao et al. are using 2D-GC-TOF-MS to characterize naphthenic acids extracted from tailing waters (137). Methylation with BF$_3$−methanol was used to improve chromatographic separation. Due to the difficulty in identifying each individual isomer, mass deconvolution software and library database searching were also important to allow detailed identifications of the toxic components. LC/MS techniques have also been explored by other researchers for identifying naphthenic acids in tailing waters. Lo et al. compared APCI-MS to ESI-MS for this purpose and found that APCI had a wider range of quantitation than ESI-MS, but ESI-MS had lower detection limits (138). These authors also fractionated naphthenic acids from tailings pond water and used Microtox to test for toxicity. Fractionation according to solubility gave fractions that did not differ much in their congener distribution or in their toxicity. However, anion-exchange chromatography fractions that had a higher proportion of multiring structures showed lower toxic potency.

ALGAL TOXINS

Algal toxins (mostly cyanobacterial toxins produced from blue-green algae) continue to be of interest in the United States and in other countries around the world. Increased discharges of nutrients (from agricultural runoff and from wastewater discharges) has led to increased algal blooms and an accompanying increased incidence of shellfish poisoning, large fish kills, and deaths of livestock and wildlife, as well as illness and death in humans. Toxins produced by these algae have been implicated in these adverse effects. Algal toxins that impact human health are generally categorized as neurotoxins or hepatotoxins that are produced from dinoflagellates, diatoms, or cyanobacteria (blue-green algae). Dinoflagellate and diatom toxins impact humans primarily through the consumption of seafood, and cyanobacterial toxins can impact humans through drinking water contamination. For example, saxitoxins, which have heterocyclic guanidine

structures, are produced by dinoflagellates and cyanobacteria and cause paralytic shellfish poisoning. Anatoxins, which have low molecular weight heterocyclic structures, are produced by cyanobacteria and are neurotoxic. Microcystins, nodularins, and cylindrospermopsin, which have cyclic peptide structures, are produced by cyanobacteria and are hepatotoxic. "Red tide" toxins, which have heterocyclic polyether structures, are produced by red tide dinoflagellates (mostly from *Gymnodinium breve*) and are neurotoxic. Microcystins are the most frequently reported of the algal toxins. The National Oceanic and Atmospheric Administration (NOAA) has a nice website that provides the structures of these algal toxins and further details (www.chbr.noaa.gov/CoastalResearch/algaeInfo.htm). Algal toxins are currently on the U.S. EPA's Contaminant Candidate List (CCL) (www.chbr.noaa.gov/default.aspx?category=mb&pageName=biotoxin). Australia also has a guideline limit for microcystin-LR of 1.3 μg/L in drinking water. Many of these toxins are peptide-related, have relatively high molecular weights, and are highly polar, which hindered their environmental measurement until the recent application of atmospheric pressure ionization techniques (ESI, APCI, and MALDI-MS).

Mass spectrometry methods that have been developed for algal toxins include LC/MS, LC/MS/MS, MALDI-MS, and ESI-FAIMS-MS. Using these methods, detection limits ranging from low nanograms to low micrograms per liter can be achieved.

Although there has been some epidemiologic evidence linking symptoms of human poisoning to cyanobacterial toxins, the presence of specific algal toxins in finished drinking water had not been proven analytically until a recent discovery of cyanobacterial toxins in finished drinking waters in Florida (*1*) and in a survey of U.S. and Canadian drinking waters sponsored by the American Water Works Association Research Foundation (AwwaRF) (*1*).

As this field of research has become more popular, more reviews have been published. Perez and Aga published a review on the advances in sample preparation, LC/MS/MS analysis, and environmental fate of microcystins in water (*139*). New extraction technologies discussed include the use of immunosorbents and molecularly imprinted polymers, which can have recoveries comparable to that of traditional SPE. Environmental fate processes discussed include biodegradation, photodegradation, and sorption. This review also provides a nice discussion of the properties of microcystins, including their chemical structures (with more than 75 structural variants identified), their sources, and how they are released into the environment. Diehnelt et al. published a review on the use of LC/MS/MS and accurate mass measurements with FT-ion cyclotron resonance-MS for measuring microcystins and for identifying new microcystins (*140*). In another review, Svrcek and Smith discuss the state of knowledge on water treatment options for reducing cyanobacterial toxins in drinking water treatment (*141*). Included in this review is a discussion of cyanobacteria and their ability to produce a variety of toxins, proposed or accepted regulatory guidelines, common detection techniques, recommendations for future research to advance the abilities of utilities to deal with these toxins, and immediate steps that can be taken for utilities to minimize human exposure to these toxins.

A number of new LC/MS methods have been published in the last two years for measuring algal toxins. Meriluoto et al. created a high-throughput LC/ESI-MS method for analyzing 10 microcystins and nodularins (*142*). This method enabled a very fast analysis time of 2.8 min/sample, which allowed the measurement of 514 samples/day. Detection limits were 50−100 pg/injection but could be lowered further to 5−10 pg through the use of newer instrumentation. Carmean used matrix solid-phase dispersion (MSPD) and LC/MS to measure microcystins in algal blooms (*143*). This procedure involved sample homogenization with C-18, washing with dichloromethane to eliminate interfering compounds, and elution with acidic methanol. Recoveries were 85−92%, and quantification limits were 1 μg/g of dry weight. Levels from 7 to 3330 μg/g were observed in culture strains and algal blooms. Bogialli et al. developed a hot water extraction-LC/MS/MS method for measuring five microcystins and nodularin in fish muscle tissue (*144*). This method was also based on MSPD, where the toxins were extracted from fish tissue with 4 mL of water acidified to pH 2 and heated at 80 °C, filtered, and injected directly onto the LC column. Recoveries ranged from 61 to 82%; lower recoveries were linked to the binding of these compounds to protein phosphatases in fish tissue. With this method, these toxins could be measured at 1.6−4.0 ng/g quantification limits. HILIC/ESI-MS was used by other researchers to measure saxitoxin, anatoxin-a, cylindrospermopsin, deoxycylindrospermopsin, microcystin-LR, and microcystin-RR in field and cultured samples of blue-green algae (*145*). However, because peak shapes were not symmetric, it was concluded that the microcystins are probably best measured with existing reversed-phase methods. Yuan and Carmichael used an unusual technique, surface-enhanced laser desorption/ionization-TOF-MS, for measuring microcystins and nodularins in water (*146*). This technique involves the capture, purification, analysis, and processing of complex biological mixtures directly onto a hydrophobic chip. With this method, 2.5 pg of microcystin-LR could be detected in 2 μL of water (equivalent to 1.2 μg/L).

New GC/MS methods are not as common but are still useful for lower molecular weight algal toxins, such as anatoxins. To that end, a new SPME-GC/MS method was developed for measuring anatoxin-a (*147*). Ghassempour et al. electrochemically coated gold wires with polyaniline and polypyrrole and used them as sorbents for SPME. GC/MS was used for detection. Calibration curves were linear over the range of 50−10 000 ng/mL, and detection limits of 11.2 ng/mL were achieved.

Interesting new occurrence and forensic studies also continue to be published. James et al. used nano-ESI with Q-TOF-MS to measure anatoxins and their degradation products in a poisoning episode of dogs who drank from a lake in Ireland (*148*). The high mass accuracy of Q-TOF-MS was important for confirming empirical formula assignments for the major product ions observed, and MS/MS was important for constructing fragmentation pathways in the identification of these toxins. Another forensic investigation in France involved the poisoning deaths of two dogs who drank from a river that had experienced cyanobacterial blooms (*149*). The dogs both experienced symptoms of vomiting, paralysis of the muscles in the hind legs, and respiratory failure before dying. In this study, LC/ESI-Q-TOF-MS/MS was used to identify anatoxin-a in the dogs' livers and in the biofilm samples

from the river, which was found to be responsible for the poisoning. This identification was particularly challenging because anatoxin-a (which is a low molecular weight, secondary amine alkaloid) has the same pseudomolecular ion as phenylalanine, which is also present in liver samples. This study also enabled the discovery of a new anatoxin-a producing algae species, *Phormidium favosum*.

Finally, brevetoxin metabolites were the focus of a new LC/ESI-MS/MS method by Wang et al. (*150*). In this study, brevetoxin-1 (the most toxic of the red tide algal toxins) and brevetoxin-2 (the most abundant of the red tide algal toxins) were incubated with rat liver hepatocytes and rat liver microsomes, respectively. Brevetoxin-1 produced two metabolites: brevetoxin-1-M2 (molecular mass of 900 Da) and brevetoxin-2-M2 (molecular mass of 884 Da). Brevetoxin-2 also produced two metabolites: brevetoxin-2-M1 (molecular mass of 912 Da) and brevetoxin-2-M2 (molecular mass of 896 Da). MS/MS enabled the metabolite structures to be proposed.

PERCHLORATE

Perchlorate has become an important environmental issue since its discovery in 1997 in a number of water supplies in the western United States. It has also recently been found in water supplies across the United States at microgram per liter levels. High quantities of perchlorate have been disposed of since the 1950s in Nevada, California, and Utah, which is believed to have contributed to much of the contamination in the western United States. However, new analyses have revealed that perchlorate contamination is not limited to the western United States; even areas such as Washington D.C. have reported perchlorate contamination, possibly caused by buried munitions. Ammonium perchlorate has been used as an oxygenate in solid propellants used for rockets, missiles, and fireworks, and there is also possible contamination that can occur through the use of fertilizers (that contain Chilean nitrate). In addition, surprising results from a new study published in 2005 indicate that perchlorate contamination can also come from natural sources, arising from atmospheric processes (*151*). Perchlorate is an anion that is very water soluble and environmentally stable. It has shown to accumulate in plants (including lettuce, wheat, and alfalfa), which could be a potential source of perchlorate exposure to humans and animals. In addition, perchlorate is not removed by conventional water treatment processes, so human exposure could also come through drinking water. Health concerns arise from perchlorate's ability to displace iodine in the thyroid gland, which could affect normal metabolism, growth, and development. Perchlorate has also been found in cow's milk, human breast milk, and human urine. Due to these concerns, the U.S. EPA has placed perchlorate on the CCL for further study. In 2004, the State of California became the first state to set a drinking water public health goal (6 μg/L), and at least seven other states have issued advisory levels ranging from 1 to 18 μg/L (*151*).

Because of perchlorate being listed on the CCL, new EPA methods have been developed, including a new IC/ESI-MS method and a new LC/ESI-MS/MS method. These methods were created to overcome matrix interferences in high ionic strength waters and also to lower detection limits to levels that are of human health concern. The only previously approved EPA method for measuring perchlorate (EPA Method 314.0, an IC conductivity method) has a minimum reporting level of 4 μg/L and is vulnerable to loss of sensitivity and false positive identifications in high ionic strength waters. The new IC/ESI-MS method (Method 330.0), Determination of Perchlorate in Drinking Water by Ion Chromatography with Suppressed Conductivity and Electrospray Ionization Mass Spectrometry (www.epa.gov/nerlcwww/ordmeth.htm), uses an O-18 labeled perchlorate internal standard, an IC suppressor column, and ESI-MS for detection (*152*). The O-18 labeled perchlorate enabled a higher degree of accuracy from both low and high ionic strength waters. Also, by monitoring selective ions specific to perchlorate, potential false positives that can occur with an IC conductivity method (due to coelution of other contaminants) were minimized. Low detection limits ranging from 0.02 (deionized water) to 0.05 μg/L (1000 ppm chloride, carbonate, sulfate) were obtained with this method. The LC/ESI-MS/MS method, Method 331.0, Determination of Perchlorate in Drinking Water by Liquid Chromatography Electrospray Ionization Mass Spectrometry (www.epa.gov/safewater/methods/sourcalt.html), is an extension of the new 330.0 method. It also uses O-18 labeled perchlorate, but it utilizes MRM with a triple quadrupole mass spectrometer, giving further selectivity to the measurement of perchlorate. This method allows 0.02 μg/L detection limits.

As mentioned earlier, there was a new discovery by Dasgupta et al. that perchlorate contamination can arise from natural sources. This was a surprising discovery and came from the observation of high perchlorate levels in groundwater from the Texas Panhandle region, where there is no historical or current evidence of the presence of rocket fuel or Chilean fertilizer sources (*151*). This perchlorate contamination is spread over 60 000 square miles, and levels of 20 μg/L are consistently found, with some measurements as high as 60 μg/L. While there were no known anthropogenic sources for the perchlorate contamination, the land had been irrigated since the 1940s, and this led the researchers to investigate potential natural sources. To this end, Dasgupta et al. demonstrated for the first time that perchlorate can readily form by a variety of simulated atmospheric processes, including by electrical discharge of chloride aerosols (with lightning) and by exposing aqueous chloride to high concentrations of ozone, which may occur in the atmosphere. Large-volume preconcentration with IC/ESI-MS was used to measure perchlorate in rain and snow samples collected from this region. Perchlorate was found in 70% of these samples at concentrations ranging from 0.02 to 1.6 μg/L. These results strongly suggest that some perchlorate is formed in the atmosphere and that a natural perchlorate background of atmospheric origin exists.

A new study has also reported perchlorate in human breast milk for the first time (*153*). In this study from Kirk et al., levels were as high as 92 μg/L in human milk collected from nursing mothers from 17 states in the United States. These levels are 20 times higher than the National Academy of Sciences committee's estimate of a safe dose (*154*). However, it is uncertain as to whether these levels cause adverse effects in infants, as the newborns were normal and had normal thyroid hormones. IC/ESI-MS was used for detection of perchlorate. Another study by Jackson et al. showed perchlorate accumulation in forage and edible vegetation, including soybean, wheat, alfalfa, cucumber,

cantaloupe, and tomato plants (155). Highest bioconcentration factors (40 to 628) were observed in soybean leaves and pods, tomato leaves, alfalfa, and wheat stems and heads. Lower bioconcentration factors (0.5–20) were seen in fruits and seeds of these plants. This study demonstrates the potential for human exposure to perchlorate through food crops.

In addition to the new EPA methods mentioned above, other new IC/MS and LC/MS have been published in the last two years. For example, Martinelango et al. reported a new IC/ion pair-ESI-MS method that provided greater selectivity and sensitivity than other single-stage MS approaches (156). For this method, a long-chain cationic agent was added postcolumn to form a gas-phase ion pair, which increased the molecular weight of perchlorate above much of the chemical noise ($m/z > 300$). Detection limits of 25 ng/L could be achieved with this method, without the need for tandem-MS. A new IC/MS/MS method was published by Krynitsky et al. for determining perchlorate in lettuce, cantaloupe, bottled water, and milk (157). An O-18 labeled internal standard of perchlorate was used, and the limits of quantification were 1.0 μg/kg for lettuce, 2.0 μg/kg in cantaloupe, 0.50 μg/L in bottled water, and 3.0 μg/L in milk. A new IC/ESI-MS/MS method was also developed by researchers at the CDC for measuring perchlorate in human urine (158). An O-18 labeled standard of perchlorate was used, and the use of MS/MS eliminated the need for sample cleanup, resulting in a rugged and rapid method capable of routinely analyzing 75 samples/day. The lowest quantifiable level (0.025 ng/mL) was sufficiently sensitive to detect perchlorate in all human urine samples evaluated.

New LC/MS/MS methods include one by Li and George for measuring perchlorate in water (159) and one by Snyder et al. for measuring perchlorate (along with bromate, chlorate, and iodate) in natural and bottled waters (160). The first method achieved 0.007 and 0.009 μg/L detection limits for deionized reagent water and synthesized reagent water, respectively. The second method achieved detection limits of 0.021 μg/L for perchlorate in water. Using this method, the authors investigated 21 commercially available bottled waters and found perchlorate in most of them, with levels up to 0.74 μg/L. Natural waters (including several rivers) and treated drinking water samples also contained perchlorate at levels up to 6.8 μg/L (for a municipal drinking water sample).

PESTICIDE DEGRADATION PRODUCTS AND NEW PESTICIDES

Herbicides and pesticides continue to be studied more than any other environmental contaminant. Lately, however, there is more of an emphasis on their degradation products, with the recognition that the degradation products (often hydrolysis products) can be present at greater levels in the environment than the parent pesticide itself (and often, the degradation product is as toxic or more toxic than the parent pesticide). For example, in a nice study by the USGS, Kolpin et al. measured 86 municipal wells in Iowa for 21 herbicide parent compounds and 24 herbicide degradates (161). The frequency of detection was 17% when only herbicide parent compounds were analyzed, but this detection frequency went up to 53% when both the parent herbicide and the degradation product were analyzed. Thus, the transport of herbicides to groundwater can be substantially underestimated when herbicide degradates are not considered. In another study,

Rebich et al. detected the herbicide degradates, metolachlor ethanesulfonic acid (ESA), deethylatrazine, metolachlor oxanilic acid, and alachlor ESA in 37–69% of the water samples collected from the Mississippi River Basin (162).

Two sets of pesticide degradation products are currently on the CCL: alachlor ESA and other acetanilide pesticide degradation products and triazines and their degradation products (including, but not limited to cyanazine and atrazine-desethyl) (www.epa.gov/safewater/ccl/cclfs.html). LC/MS and LC/MS/MS are now becoming common-place techniques for measuring these pesticide degradates, which are generally more polar than the parent pesticides, making LC/MS ideal for their detection.

As in studies of other emerging contaminants, TOF-MS (and Q-TOF-MS) is being used more and more to identify new transformation products. Studies by Ibanez et al. (163), Thurman et al. (164), and Ibanez et al. (165) are examples. Ibanez et al. used LC/Q-TOF-MS to determine the structures of photodegradation and biotransformation products of the insecticide diazinon (163). The mass accuracy of TOF-MS allowed the assignment of empirical formulas, and MS/MS allowed for further characterization of the compounds and differentiation of isomers. In the paper by Thurman et al., LC/TOF-MS and LC/ion trap-MS were used together to determine the structures of unknown pesticides and their metabolites on tomato skins (164). The accurate mass capability of TOF-MS was also important for generating possible empirical formulas and final structures in this study. In another study, Ibanez used LC/Q-TOF-MS to elucidate the transformation products of triazine herbicides in water after UV exposure (165).

Four reviews published in the last two years are worthy of note. Pico et al. reviewed the environmental and food applications of LC/tandem-MS for pesticide analysis (166). In this review, different MS instrumentation was compared for pesticide analysis, and advantages and drawbacks of the different techniques were discussed. Sample preparation techniques, as well as examples of applications, were also presented. Hernandez et al. reviewed the applications of LC/MS for determining pesticides in biological samples (167). Discussions included whole blood, plasma, serum, and urine samples, and different MS techniques were compared. Budde published a comprehensive review on herbicides registered by the U.S. EPA, which included compounds in 21 categories, according to their general chemical structures or common structural group (168). The herbicides were discussed in terms of their structures, their EI mass spectra, amenability to separation and measurement with GC, LC, and CE combined with MS. Finally, Medana et al. reviewed the use of LC/tandem-MS for investigating pesticides and their degradation products (169).

Several nice occurrence studies have investigated pesticide degradation products. In one by Kolpin et al., concentrations of glyphosate and its degradate aminomethyl phosphonic acid (AMPA) in U.S. streams were determined (170). Glyphosate (also known as Roundup in one commercial product) is a nonselective, broad-spectrum herbicide and is now the most widely used herbicide in the world. Because glyphosate and AMPA are highly polar and water soluble, they are ideally suited for measurement by LC/MS. This method used precolumn derivatization with 9-fluorenylmethyl chloroformate, followed by automated on-line SPE and LC/MS measurement. Glyphosate or its degradate AMPA were commonly detected in streams and wastewater

treatment plant effluents. The results also document the contribution of wastewater treatment plant effluents to stream concentrations of glyphosate and AMPA. Overall, AMPA was detected much more frequently (67.5%) than glyphosate (17.5%). Sancho et al. used ion pair LC/ESI-MS/MS to determine cyromazine and its metabolite melamine in chard samples (*171*). Quantification limits were 0.05 μg/kg, and detection limits were 0.01 mg/kg.

Smalling and Aelion used GC/MS to investigate biological and chemical transformation products of the herbicide atrazine in coastal sediments (*172*). Degradation products—deethylatrazine and deiosopropylatrazine—were found in these samples, but were not found at significant levels and were not believed to be an ecological threat. However, a new secondary metabolite—methylated atrazine—was formed from atrazine in the sediments and was present at significant levels.

While pesticide parent compounds are not necessarily the focus of this section, an important paper by Sannino et al. on determining 24 new pesticides with LC/MS/MS is worth mentioning (*173*). These new pesticides include azoxystrobin, trifloxystrobin, kresoxim-methyl, fenazaquin, indoxacarb, fenothiocarb, furathiocarb, benfuracarb, imidachloprid, dimethomorph, fenpyroximate, hexythiazox, tebufenpyrad, tebufenozide, difeconazole, fenbuconazole, flusilazole, paclobutrazol, tebuconazole, tetraconazole, bromuconazole, etofenprox, fenhexamid, and pyridaben. They were measured in apple puree, concentrated lemon juice, and tomato puree. A miniaturized extraction—partition procedure required only small amounts of nonchlorinated solvents, and LC/ESI-MS/MS allowed 1–20 μg/kg detection limits.

Interesting human exposure studies were also published. Hernandez et al. used LC/ESI-MS/MS to estimate exposure to organophosphorus pesticides by determining their metabolites in urine (*174*). Sub-ppb levels of the metabolites could be measured. Finally, Corrion et al. used GC/EI-MS to measure prenatal exposure to several classes of pesticides and their metabolites (*175*). In this study, maternal and cord whole blood were investigated, and results showed detectable levels of propoxur, 3-phenoxybenzoic acid, and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (*p,p′*-DDE) in maternal and umbilical cord samples collected from mothers and infants from a region in the Philippines that has high pesticides use.

CHIRAL CONTAMINANTS

The last two years has continued to see growth in the use of chiral chromatography with MS. Chiral chromatography is used to analyze individual chiral isomers, which are very similar chemically, but can behave differently in the environment and in biological systems. New research on chiral pesticides and chiral UV filters is reported in this review. For chiral pesticides, typically, one form is active against the insects, weeds, or other pests that the pesticide is designed to attack, and the other form is inactive. Likewise, in the environment, one form can be actively degraded by microbes, and the other form can accumulate. It was not until recent developments allowed the separation and low-level detection of these isomers that their environmental behavior could be studied. However, early research is showing that the environmental behavior of chiral compounds is not straightforward—it is not always possible to predict enantiospecific transformations. Microbial populations in environmental matrixes can change, and

even reverse, the enantiomeric ratios (so microbial processes may not always show selective degradation of the same enantiomer). Some environmental processes are not enantioselective toward a particular chemical, even if microorganisms are involved. Sometimes microbial degradation rates are sufficiently rapid for both enantiomers, so that enantioselective degradation is not important. Some compounds are degraded much faster chemically (abiotically) than microbially, so that enantioselective degradation is not important, and sometimes enantiomerization can occur, where one enantiomer is microbially converted to the other (*176*).

The ability to separate enantiomers and produce a single enantiomeric isomer has not been lost on pesticide manufacturers. This ability has allowed manufacturers to sell a new, patented enantiomeric form of a pesticide, creating new markets for their products. The development of enantiomerically enriched pesticides may actually benefit the environment, as less material could potentially be applied to crops, less may be accumulated in the environment, and there may be fewer unintended side effects on nontarget species. However, more research is needed to make this determination.

Most research to-date has investigated chiral profiles in surface waters, soil, and vegetation. The most commonly used analytical techniques to separate and measure chiral isomers include the use of chiral columns with GC and LC (often including the use of mass spectrometry). CE is also often used. Chiral selectors now include cyclodextrins, proteins, crown ethers, polysaccharides, polyacrylamides, polymeric chiral surfactants, macrocyclic antibiotics, and ergot alkaloids. Cyclodextrins still remain the most popular chiral selectors for environmental applications.

Growing interest in the study of chiral contaminants is evidenced by the increasing number of reviews published in the last two years. Garrison published a nice review in early 2006 on chiral compounds in the environment (*176*). Chiral pesticides were covered in detail, with discussions of the enantiomer-specific fate and enantiomer-specific effects of chiral pesticides. In addition, other chiral environmental contaminants were mentioned, including DBPs, PCBs, flame retardants, and pharmaceuticals. Müller and Kohler published a similar review on the metabolism and fate of chiral pollutants (*177*). In this review, the fate (mostly by biodegradation) of chiral phenoxyalkanoic acid herbicides, acetamides, organochlorines, and linear alkylbenzenesulfonates was discussed. The difficulty in predicting which enantiomer may be enriched was also addressed, as well as racemization and enantiomerization processes that can complicate interpretation of data. In another review, He and Beesley reviewed applications of chiral-GC and described different chiral stationary phases used with chiral-GC/MS (*178*). Ward published a 2004 *Analytical Chemistry* review on chiral separations, which covered recent developments from 2002 to 2004 (*179*). This review provided details on the types of chiral phases used for separations, various separation techniques (including LC, GC, CE, microchip-CE, supercritical fluid chromatography, and thin-layer chromatography (TLC)) and applications to the measurement of a number of different chiral compounds.

Several interesting environmental studies have also been published in the last two years. Buser et al. investigated the stereoisomer composition of the chiral UV filter 4-MBC in the aquatic environment (*180*). 4-MBC exists as (*E*)- and (*Z*)-isomers

(like cis/trans isomers), both of which are chiral. Chiral-GC/MS was used to determine the stereoisomers. Technical material and a commercial sunscreen lotion contained mostly the (E)-isomer, with a racemic composition of the enantiomers (R/S = 1.00). Untreated wastewater showed a nearly racemic composition, suggesting that most if not all commercial 4-MBC is racemic. Treated wastewater showed a slight excess of (R)- or (S)-stereoisomers, indicating that some enantioselective biodegradation is occurring. A slight enantiomeric excess was also observed in Swiss lakes, rivers, and fish. Mattina et al. used chiral-GC/MS to investigate the plant uptake and translocation of highly weathered, soil-bound chlordane in field studies (181). Component fractions and enantiomer fractions of both chiral and achiral chlordanes were followed through soil, root, xylem sap, and aerial tissue compartments of zucchini plants. Data showed that the chlordane residues translocated enantioselectively from the soil matrix into and through the plant environment with genera-specific patterns. The determination of chlordanes for the first time in the xylem sap of plants grown in contaminated soil confirms the presence of a soil-sequestered and highly hydrophobic organic contaminant within the aqueous plant environment.

Hamed et al. used multidimensional-GC/ECNI-MS to measure chiral toxaphene congeners in laying hens and eggs from hens that were exposed to toxaphene-contaminated food (182). Significant enantiomeric differences were observed. Interestingly, some of the toxaphenes applied as racemates could only be found as single enantiomers at the end of the feeding program, which demonstrated that the metabolism was enantiomerically specific. Toxaphene elimination by fish was the focus of another study by Maruya et al. (183). Chiral-GC/ECNI-MS was used to measure the individual enantiomers in the fish, and enantioselective elimination of metabolites was observed. In another study, Larsson et al. measured PCB and DDE chiral metabolites in gray seals from the Baltic Sea that were naturally exposed to organochlorines through their food (184). The enantiomeric specificity was equal in all tissues measured (including the liver, lung, and adipose tissues). Finally, Liu et al. examined the occurrence and enantioselectivity of a number of synthetic pyrethroid and organophosphate insecticides in the environment (185). Chiral-GC/MS was used for measurements. Dramatic differences between enantiomers were observed in their acute toxicity to freshwater invertebrates, and in field sediments, specific enantiomers of cis-bifenthrin and cis-permethrin were preferentially degraded, resulting in the enrichment of one enantiomer.

MICROORGANISMS

Outbreaks of waterborne illness in the United States and other parts of the world (including Escherichia coli-induced gastroenteritis in Walkerton, Ontario, in 2000, cryptosporidiosis in Milwaukee in 1993, and cholera in Peru beginning in 1991) have necessitated improved analytical methods for detecting and identifying microorganisms in water and other environmental samples. Mass spectrometry had played a minor role in the past through the use of pyrolysis-GC/MS, but is now playing a more important role, with increased research using MALDI-MS and ESI-MS techniques, which can be used on whole or treated cells. These MS methods offer a very rapid analysis time (~10 min) and specific information that can be used to distinguish different

strains of the same organism.

Bothner and Siuzdak reviewed the use of ESI-MS for analyzing the mass, structure, and viability of viruses (186). These authors discuss the technical challenges in analyzing viruses (and other microorganisms), including the limitation of the mass range for most ESI instruments and the small signal generated by slow-moving megadalton particles. The operation of a quadrupole mass analyzer in the radio frequency mode was mentioned as an initial means to overcome the mass analyzer limit, and the later development of ESI-TOF-MS was a more optimal way of measuring an intact virus. In addition, ESI-ion mobility spectrometry (IMS) can be used to determine the cross-sectional area of an ion, which can confirm that the virus remains intact during the ESI-MS analysis and that no large-scale disruption of the tertiary or quaternary structure occurred during desolvation and ionization.

While research groups are still analyzing whole-cell bacteria and other microorganisms by ESI-MS and MALDI-MS, advantages are being recognized in digesting the proteins in microorganisms prior to ESI- or MALDI-MS analysis (187). Intact protein (and microorganism) analysis offers the advantage of minimal sample preparation, but the higher masses hinder mass accuracy, resolution, and sensitivity (187). On the other hand, digestion can be performed in situ, and the lower mass protein biomarker fragments can then be more easily analyzed and can be used to identify the microorganism. Fenselau's research group is demonstrating the utility of this approach, with two recent papers on the MALDI-MS analysis of Bacillus spores (187) and enterobacteria (188). Results from these studies showed that the peptides identified from the microwave-assisted hydrolysis of Bacillus spores (187) and the trypsin digestion of proteins in enterobacteria (188) were unique to these organisms and could be used for their identification. In another study utilizing tryptic digestion (and LC/ESI-MS/MS), Dworzanski et al. were able to classify bacteria according to their strain level (189).

Among the whole-cell microorganism studies still being published, Wunschel et al. reported an interlaboratory comparison of the analysis of bacteria with MALDI-TOF-MS (190). Automated data processing and analysis algorithms were used, and fingerprints from the three laboratories were compared. Despite different laboratory conditions and instruments, a collection of common ions was observed reproducibly across the three laboratories when a standard protocol was followed. Within a given laboratory, correct bacterial identifications were achieved for 90−100% of the test spectra. In another study using whole-cell analysis and "top-down proteomics" (where MS/MS is used to generate fragment ions for identification), Demirev et al. applied MALDI-TOF-MS to identify intact Bacillus spores (191). In this study, small acid-soluble spore proteins were used to identify the species either in pure form or in mixtures. Mazzeo used MALDI-TOF-MS to discriminate 24 bacterial species and to identify E. coli O157: H7 for food-borne applications (192).

The risk of airborne pathogens in wartime or bioterrorism situations has also prompted new work on fieldable instrumentation that can be used for rapid detection. Snyder et al. created a pyrolysis-GC-IMS-TOF-MS briefcase system to detect and classify deliberately released bioaerosols in outdoor field scenarios (193). The bioaerosols included Gram-positive and Gram-negative

bacteria, MS-2 coliphage virus, and ovalbumin protein species. Microorganism components produced by pyrolysis and detected by IMS were identified by their GC retention times and EI mass spectra (with library database matching). Van Wuijckhuijse et al. developed a new aerosol TOF-MS instrument for the near-real-time analysis of single bioaerosol particles (194). This system was combined with laser-induced fluorescence selection and MALDI-MS, and aerosolized proteinaceous material up to 20 000 Da (which is in the range of molecular masses of marker ions of bacteria) could be measured.

Additional advances have been made to address the problems with ion suppression that can occur with MALDI-MS of whole cells in complex, real-world mixtures. For example, Lin et al. developed a method for extracting and concentrating trace amounts of bacteria from complex microorganism mixtures, using vancomycin-bound magnetic nanoparticles (195). These nanoparticles were used as affinity probes to selectively trap Gram-positive pathogens from sample solutions using a magnetic field. This approach reduced the interference of protein and metabolite signals in the MALDI mass spectra obtained.

Finally, mass spectrometry continues to be used to probe the structures of microorganisms. Lee et al. used TLC, MALDI-TOF-MS, and ESI-MS/MS to identify a lipid from the virulent *E. coli* O157:H7 (196). TLC was used to isolate the lipid from the organism, after which it was reextracted with chloroform–methanol and analyzed.

ARSENIC

Unlike many other contaminants that are anthropogenic, arsenic contamination of waters generally comes from natural sources, through the erosion of rocks, minerals, and soils. Arsenic contamination of drinking water in Bangladesh and India has become a highly recognized problem, but natural arsenic contamination also affects several regions of the United States and other parts of the world. For several years, the U.S. EPA has conducted research on arsenic (occurrence, health effects, bioavailability) and, in 2002, lowered the maximum contaminant level (MCL) from 50 to 10 μg/L, which is believed to be a level that would better protect human health (www.epa.gov/safewater/arsenic.html). Drinking water systems must comply with this new standard by January 23, 2006. The World Health Organization (WHO) also has this same standard of 10 μg/L in drinking water. The general toxicity of arsenic is well known, but studies have also linked long-term exposure of arsenic (at lower, nontoxic levels) to a variety of cancers in humans. In addition, there are recent reports of excess risk of spontaneous abortion, stillbirth, and neonatal death.

Mass spectrometry is commonly used to measure the individual arsenic species, with GC/MS, LC/ESI-MS, LC/ICP-MS, and IC/ICP-MS techniques used. Different arsenic species have different toxicities and chemical behavior in aquatic systems, so it is important to be able to identify and quantify them. More than 20 arsenic species are present in the natural environment and in biological systems. These include arsenite, arsenate, monomethylarsonic acid, monomethylarsonous acid, dimethylarsinic acid, dimethylarsinous acid, trimethylarsine oxide, trimethylarsine, arsenobetaine, arsenocholine, tetramethylarsonium ion, dimethylarsinoyl ethanol, and arsenosugars (197).

The water analysis review from 2005 (2) covers the development of new methods for measuring arsenic species in water, as well as important occurrence studies that have been recently conducted. While it is widely known that arsenic contamination affects millions of people worldwide, the mode of action by which arsenic causes health effects is not clear. To that end, this review will cover some exciting new biological studies of arsenic that are being conducted to understand the mode of action of arsenic and its metabolism in health effects studies. Le et al. published an excellent review of arsenic speciation that discusses in detail the metabolism of arsenosugars that are ingested from the consumption of seaweed, mussels, oysters, and clams (197). The use of LC/ICPMS and ESI-MS for studying arsenic metabolites and for understanding arsenic binding to proteins in vivo is outlined. In another interesting study, Lu et al. address the differences observed in toxicity experiments with rats versus humans (198). A puzzling finding has been that rats show a longer retention time in the blood for arsenic, whereas arsenic is rapidly cleared from human blood (half-life of 1 h). These biological differences are not understood and can limit the use of animal models for understanding human health effects. Lu et al. used gel filtration chromatography with ICPMS and nanospray-ESI-MS to test their hypothesis that rat hemoglobin binds directly to trivalent arsenic compounds, resulting in the retention of arsenic in the red blood cells. Results from in vivo experiments of rats fed an arsenic-supplemented diet showed that the arsenic was mostly in the protein-bound form. In vitro experiments with isolated human and rat red blood cells showed that 15–30 times more arsenic species were bound to the hemoglobin of rats than to the hemoglobin of humans. Further, the binding affinity of arsenic to rat hemoglobin was shown to be 3–16 times stronger than to human hemoglobin. These results suggest that the stronger binding affinity of these arsenic species to rat hemoglobin is responsible for the accumulation of arsenic in rat blood.

Dopp et al. used ICPMS to investigate the biological uptake of arsenate, arsenite, monomethylarsonic acid, monomethylarsonous acid, dimethylarsinic acid, dimethylarsinous acid, and trimethylarsine oxide (199). Results indicated that the uptake of arsenic compounds was highly dependent on the cell type. It was proposed that arsenic-induced genotoxic effects observed in fibroblasts are due to the high uptake of arsenicals into this cell type, which may explain the high susceptibility of skin fibroblasts to arsenic exposure. Kile et al. used ICPMS to investigate the uptake of arsenic in people with different gene polymorphisms (200). In this study, it was found that people with GSTT1-null genotypes had significantly more arsenic in their toenails as compared to GSTT1 wild-type individuals (for people exposed to the same concentrations of arsenic in their drinking water). This was an important finding since toenail arsenic concentrations are frequently used as biomarkers of arsenic exposure.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. EPA.

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