Emerging contaminants in the environment

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Introduction

This chapter explores the use of mass spectrometry and its application to emerging contaminants (ECs) in the environment; such classes of compounds as organometallics, pharmaceuticals/drugs, nanomaterials, and dispersants (surfactants). Table 1 shows the variety of ECs that are available, however this table should not be thought of as an exhaustive listing, but as a helpful reference for this chapter.

What does "emerging contaminant" mean? For example, in 3000 B.C. lead was co-extracted with silver from silver mines in Anatolia and subsequently discarded. To those who lived in the vicinity of the mine, and whose water and food sources were contaminated with leftover lead, the lead may well have been considered an emerging contaminant. Skipping forward five thousand years, at the beginning of the industrial age the United States (US) Tariff Commission published (1918-1919) that the combined production total of synthetic organic chemicals was nearly 800 million pounds (USTC 1919). The finished products were diverse: dyes, color lakes, photographic chemicals, medicinals, flavors, perfume materials, synthetic phenolic resins, synthetic tanning materials, and explosives (USTC 1919). In contrast, in 2007 the US production volume of organic synthetic chemicals was nearly 27 trillion lbs (USEPA 2008). Should all 27 trillion lbs be considered ECs? The answer lies within our modern concept of an "emerging contaminant". The idea of what is an EC really didn't take hold until it was recognized that not every chemical that is manufactured is a "good" chemical for the environment. In 1962 the publishing of Rachel Carson's seminal book "Silent Spring" (Carson 1962) brought forth to the attention of the American public, and the global

community, the hidden dangers of what was thought to be a "good" chemical, dichlorodiphenyltrichloroethane (DDT). DDT was great for public health; it killed malaria-bearing mosquitoes, squelched infestations of bedbugs, and had other beneficial properties for human well-being. However, now it is widely known and accepted that DDT was responsible for the near extinction of the bald eagle, and other birds of prey, and DDT was officially banned from use in the US on June 14, 1972 (http://www.epa.gov/history/topics/ddt/01.htm). Although DDT has not been used for nearly 40 years in the US, the use of mass spectrometry has determined that there are still residual amounts of DDT and its breakdown/transformation products, dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), to be found in the environment (McMahon, Dennehy et al. 2006; Lubick 2007).

Many synthetic organic chemicals have brought positive benefits to humankind, and yet they can have unintended negative consequences for both human and environmental health. For example, organotins have a wide variety of beneficial applications in the modern world. Organotins are used as molluscides in nautical paints; as fungicides in agriculture, indoor/outdoor house paints, and indoor flooring and wallpaper; and as industrial polymerizers in plastics (Hoch 2001; Appel 2004). However, they have been implicated as endocrine disruptors, neurotoxins, inducing diabetes, and other unintended negative effects (Huggett, Unger et al. 1992; Eskes, Honegger et al. 1999; Appel 2004; Grun and Blumberg 2006; Grote, Hobler et al. 2007; Grote, Hobler et al. 2009; Moser, McGee et al. 2009; Hobler, Andrade et al. 2010). The different organotins have varying toxicity levels, therefore it is important to know which organotin

47 is found. However, analyzing just for total tin does not deliver the specificity necessary
48 to determine the organic moieties of tin; only through the use of mass spectrometry,
49 coupled to chromatography, can the various organometallics be distinguished from each
50 other (Jones-Lepp, Varner et al. 1999; Morabito, Massanisso et al. 2000; Moreno,
51 Pacheco-Arjona et al. 2006).
52
53 Another example of emerging contaminants are the use of pharmaceuticals for the
54 treatment of harmful diseases in both humans and animals and their unintended release
55 into the environment (Ankley, Brooks et al. 2007; Kemper 2008). For example, the
56 introduction and use of antibiotics in the 20th Century has led to a decrease in mortality
57 from common bacterial infections. However, it is now recognized that the increasing use
58 of human and veterinary antibiotics can lead to an increase in antibiotic resistance in the
59 environment, an unintended consequence from the use of this class of beneficial
60 pharmaceuticals (Guardabassi, Wong et al. 2002; Schwartz, Kohnen et al. 2003; Da
61 Silva, Tiago et al. 2006; Auerbach, Seyfried et al. 2007; Kim and Aga 2007; Schlüter,
62 Szczepanowski et al. 2007; Rosenblatt-Farrell 2009; Szczepanowski, Linke et al. 2009;
63 Zhang, Marrs et al. 2009). In a seven year field study Kidd et al. (2007) demonstrated
64 how the use of a beneficial drug can have unintended negative environmental
65 consequences (Kidd, Blanchfield et al. 2007). In this study they spiked a small isolated
66 lake with low levels of the synthetic estrogen used in birth-control pills [17β-
67 ethynylestradiol (EE2)]. Within seven years they demonstrated that even very low levels
68 of the synthetic estrogen caused an ecologic collapse of native fish populations to near
69 extinction levels (Kidd, Blanchfield et al. 2007). From the very beginning mass

spectrometry has played an important role in the detection of pharmaceuticals in the environment leading to their being classified as ECs (Watts, Crathorn et al. 1983; Ternes 1998; Daughton and Ternes 1999; Daughton 2001; Daughton and Jones-Lepp 2001).

Newer chemical materials are constantly being introduced into production for consumer use, most recently are anthropogenically engineered nanoparticles. Although naturally occurring nanoparticles have always been around, created either by forces of nature (e.g., volcanoes) or incidentally (e.g., emissions from combustion sources), anthropogenically engineered nanomaterials are recent inventions (Owen and Handy 2007; Lubick 2008; Farré, Gajda-Schrantz et al. 2009). These nanomaterials can be considered as ECs, and are engineered from nanometallic (e.g., silver, gold, iron) and nanocarbon (e.g., fullerenes) materials that are sized between 1 nm and 100 nm. The Woodrow Wilson Institute since 2006 has kept an on-line database of the number of consumer nanomaterials products currently being offered on the market (Woodrow 2010). The number of nanomaterial-containing products has grown substantially from 212 products listed in 2006 to 1015 products as of August 2009 (Woodrow 2010). The majority of these nanoproducts contain nanosilver, followed by nanocarbon materials. Nanomaterials will have far-reaching benefits and subsequent consequences, positive and negative, with their use (Colvin 2003; Owen and Handy 2007; Klaine, Alvarez et al. 2008).

Lastly, in this chapter we will explore the use of dispersants, and the role of mass spectrometry in aiding crisis response strategy during a major environmental crisis. In

the summer of 2010 an undersea oil well (Deepwater Horizon) in the Gulf of Mexico failed. Millions of gallons of oil leaked into the Gulf of Mexico from April 2010 until July 15, 2010, when the well was capped off. In an effort to stem the negative consequences of this much oil being released into the ocean ecosystem nearly 2 million gallons of dispersants had been deployed as of December 1, 2010 through aerial spraying, and underwater deployment, over the areas affected by the spill (http://www.restorethegulf.gov/release /2010/12/01/operations-and-ongoing-response-december-1-2010). At this time the consequences of the use of this amount of dispersant on an ocean ecosystem is unknown, and only future observations will determine if the use of dispersants was beneficial or harmful, or somewhere in-between.

Mass Spectrometry for the Analysis of Emerging Contaminants

The majority of detection techniques for ECs are mass spectrometry based. This is due to the reality that most environmental matrices are complex, and only the mass accuracy and specificity given by mass spectrometry can overcome the large amounts of interferences found in real-world matrices. For example, one of the first reports of estrogens found in the environment used HPLC-fluorescence detection, but the authors reported many polar interferences in the estrogen-containing fraction, making identification difficult (Snyder, Keith et al. 1999). Later work, by the same principal investigator (Snyder) utilized the mass accuracy and specificity of a mass spectrometer detector for the same analytes, plus they were able to characterize other pharmaceuticals in the same lake water matrix (Vanderford, Pearson et al. 2003).

There are a variety of mass spectrometers that are being used today as detectors, and the majority are coupled either to gas chromatographs (GCs) or liquid chromatographs (LCs). There are quadrupole mass spectrometers (MS), ion traps (ITMS), time-of-flight mass spectrometers (TOFMS), triple quadrupole mass spectrometers (QqQ), magnetic sector mass spectrometers, and recently orbitrap mass spectrometers. Which type of mass spectrometer to use for determining ECs in environmental matrices depends upon: type of separation technique chosen (GC or LC); mass information wanted; mass accuracy required; and specificity needed. The reader is referred to several mass spectral references for gaining a better understanding of the beginnings and basics of mass spectrometry (McLafferty 1980; Busch, Glish et al. 1988; Barceló 1996; Grayson 2002; Herbert and Johnstone 2003).

Gas chromatography-mass spectrometry. In general, chemicals that vaporize at < 300 °C, and therefore are chemically stable up to that temperature, can be measured by gas chromatography/mass spectrometry (GC/MS). Unlike non-specific detection techniques (e.g., flame ionization detector (FID), UV/diode array detector (DAD), or fluorescence), GC/MS offers the ability to produce multiple fragment ions (via electron impact ionization) from an analyte, giving the chemist an unequivocal identification technique.

ECs that are polar, and/or thermally labile need to be derivatized in order to pass through a GC, and most early attempts to identify these ECs (e.g., organometallics, pharmaceuticals) in the environment used derivatization (Kelly 2000; Moeder, Schrader

et al. 2000; Morabito, Massanisso et al. 2000; Ternes, Andersen et al. 2002). For pharmaceutical ECs, there are typically two methods of derivatization that are used to methylate the H-acidic functional groups of the e.g., -COOH and -OH groups; diazomethane, and trimethylsilyl (TMS) derivatization (Ternes 1998; Moeder, Schrader et al. 2000; Jones-Lepp, Alvarez et al. 2006). For organometallic ECs, the most common derivatization methods are either hydride generation, alkylation by Grignard reagents, or the use of sodium tetraethylborate ($NaBEt_4$) (Morabito, Massanisso et al. 2000). Derivatization methods have disadvantages. For example, incomplete derivatization can occur leading to lower recoveries, and subsequent underestimation of contamination. More specifically, the use of diazomethane is not a preferred derivatization method due to its dangerous properties (toxicity and explosivity). The use of TMS, while not "dangerous", can lead to the formation of mono- and di-TMS derivatives, which can subsequently cause interferences with identification and quantitation. Because of the limitations of derivatization, there is an increasing trend to use LC/MS as a determinative method in analyzing for polar, non-volatile, and/or thermally labile ECs in environmental matrices.

Liquid chromatography-mass spectrometry. As discussed in the previous section conventional GC/MS methods have limitations as to the types of analytes that are amenable to that detection technique. Many ECs are polar, thermally instable, hydrophobic, and have low volatility, making them ideal candidates for LC/MS. The coupling of LC to MS has been utilized for over 30 years (Niessen 2006). Briefly, the liquid mobile phase of the LC is nebulized, charged, and directed into an MS source

[most LC to MS coupling is via electrospray ionization (ESI)]. The MS source is at atmospheric pressure, and through various combinations of heated capillaries (e.g., ion cones, hexapoles, quadrupoles, and ion filters) the charged analytes are directed into the high vacuum range of the mass spectrometer detector region. One of the unique aspects of LC/MS is that the technique usually only creates a single ion in the source, allowing for identification of the molecular weight of a compound. The ion created is typically the protonated molecule, $(M+H)^+$, in the positive ionization mode, or the molecule minus the hydride ion $(M-H)^-$, in the negative ionization mode. However, this positive aspect can also be a limitation, for with only one ion for identification it would be easy to misidentify analytes in complex environmental matrices. For example, methamphetamine ($C_{10}H_{16}N$, m/z 149.23 Da, CAS 537-46-2) and N,N'-dimethylphenethylamine ($C_{10}H_{16}N$, m/z 149.23 Da, CAS 1126-71-2; DMPEA; industrial chemical used as a flavoring agent) are isobaric ions of each other, both have exactly the same molecular mass (m/z 149.23), but are slightly different in chemical structure. Therefore an analyst must go to a more specific mass spectral identification technique, referred to as tandem MS, or MS/MS techniques. This is a technique whereby a precursor ion is formed in the LC/MS source [typically the $(M+H)^+$ or $(M-H)^-$ ion], the ion is energized and collided (collision induced dissociation – CID), either in a triple quadrupole, ion trap, or a magnetic sector mass spectrometer region, and in so doing produces product ions. Product ions typically involve the loss of various functional groups from the analytes, for example $(M+H-OH)^+$ or $(M+H-CH_3)^+$. However, even using MS/MS techniques false identification is still possible. In the case of methamphetamine and DMPEA, when using CID they both form unique predominant

product ions, m/z 119 $(MH–CH_3NH_2)^+$, and m/z 105 $(MH\text{-}NH(CH_3)_2)^+$, respectively. Both compounds form m/z 91 as a secondary product ion, but through different pathways. Such that, if a researcher choses to monitor mass m/z 91, instead of m/z 119, for methamphetamine (and there are those who have reported doing so in the literature) then a false positive for methamphetamine could occur. Figure 1 is a mechanistic rationale for support of this hypothesis. <fig 1 insert chemical structure meth/DMPEA pathways>. Another example is MDMA ($C_{11}H_{15}NO_2$, mw 193.25 Da, CAS 69610-10-2) vs. caffeine ($C_8H_{10}N_4O_2$, mw 194.19 Da, CAS 58-08-02). While MDMA and caffeine have different molecular weights they have overlapping product ions (mass m/z 163), but different precursor to product pathways. MDMA with a molecular weight of m/z 193, in the ESI source forms m/z 194, $(M+H)^+$, and produces mass m/z 163.0, $(MH–CH_3NH_2)^+$, as the predominant product ion using MS/MS. Caffeine has a molecular weight of m/z 194 (one amu different from MDMA), and forms m/z 195, $(M+H)^+$ in the positive mode, and m/z 138, $(MH\text{-}CH_3NCO)^+$ is the predominant product ion formed under CID, with mass m/z 163 also formed, but less abundantly. Therefore, if an analyst were to monitor the m/z 163 ion channel, and detected m/z 163, near or at the same retention time as caffeine, they might misidentify that compound as MDMA, when in fact it is caffeine. Figure 2 is a mechanistic rationale for support of this hypothesis. <fig 2 insert chemical structure MDMA/Caffeine pathways>.

When using LC/MS techniques for identifying known, and unknown, chemicals, it cannot be emphasized enough that the analyst must use a LC/MS/MS technique in

order to accurately identify the unknown analytes, and it is important that the proper product and transition ions are chosen to ensure specificity and accuracy.

Organometallics. Organometallic compounds are used daily in a variety of consumer, agricultural, and industrial products. Many of these synthetic compounds are important in medicine (e.g., organoferreous and organoplatinum as anti-tumor agents; organoboron in neutron capture therapy), household products (dibutyltin, dimethyltin, octyltin in plastic formulations), agriculture (triphenyltin, fungicide; cacodylic acid as a contact herbicide; phenylarsonic acids as animal growth promoters), and in the shipping industry (tributyltin and triphenylboron as anti-mulluscides) (Huggett, Unger et al. 1992; Craig 2003; Jones-Lepp and Momplaisir 2005; Allard, Passirani et al. 2008; Oudijk 2010). Biological transformations of metal or metalloid species contribute to organometallic compounds in the environment, as well as anthropogenic activities, such as mining and the energy industry (e.g., methylmercury and alkyllead) (Tessier and Turner 1995). Determining individual chemical species rather than total element concentrations is important due to differences in toxicological and biochemical properties of organometallic compounds (Tessier and Turner 1995; Newcombe, Raab et al. 2010). For the capability to speciate individual organometallics hyphenated mass spectral techniques are essential.

Organotins. As mentioned previously organotin compounds can elicit a wide range of endocrine- and nervous-system effects, depending on the nature and number of alkyl groups bonded to the tin atom. Therefore, it is important to be able to determine

the specific organotin structure and not just the total tin available. Tin, ^{120}Sn, has 10 stable isotopes, which makes a unique GC-MS and LC-ESI-MS mass spectral pattern, helping in the distinctive identification of organotin compounds in environmental samples. Most methods for detecting organotins are GC-MS based, which means that derivatization must happen before detection. In Thomaidis et al. seawaters were collected, adjusted to pH 5, and the organotins were extracted and derivatized with a sodium tetraethylborate (STEB) and hexane solution (Thomaidis, Stasinakis et al. 2007). The derivatized extracts were then analyzed by GC-MS, where the instrumental limit-of-detection (LOD) for the butyltins was around 2 pg injected (1 µL injection = 2000 ng L^{-1}) as tin. For phenyltins the LOD was lower, particularly for triphenyltin (LOD=1 pg) (Thomaidis, Stasinakis et al. 2007). Segovia-Martínez et al. were able to obtain even lower LODs for the organotins, from 0.025 ng L^{-1} for tributyltin and diphenyltin to 1 ng L^{-1} for tetraethyltin (Segovia-Martínez, Bouzas-Blanco et al. 2010). Their method was based on *in situ* ethylation and simultaneous headspace-solid-phase microextraction (HS-SPME) and GC–MS analysis (Segovia-Martínez, Bouzas-Blanco et al. 2010). Figure 3 shows the mass spectra obtained, by for tetraethyltin, tributyltin, diphenyltin, and triphenyltin with this method. In each of the spectra are the characteristic isotope patterns for ^{120}Sn. **<insert figure 3 segovia-marinez>** Because their method relied on derivatization with STEB the spectral patterns show the characteristic ions of the non-derivatized ions and the ethylated ions. For example, in the tributyltin spectrum the masses representing the non-derivatized organotin ions are: m/z 179 ($SnBuH_2$) and m/z 291 ($SnBu_3$); and the ethylated organotins are: m/z 151 ($SnEtH_2$), m/z 207 (SnEtBuH), m/z 235 ($SnEt_2Bu$) and m/z 263 ($SnEtBu_2$) (Segovia-Martínez, Bouzas-Blanco et al.

253 2010). Organotin methods for other matrices besides waters have been recently
254 developed. Organotin compounds in netted dog whelk (*Nassarius reticulates*) samples
255 were quantified by using a SPE extraction, followed by STEB derivatization, and analysis
256 by GC-MSD (Sousa, Laranjeiro et al. 2009). Kannan et al. (Kannan, Takahashi et al.
257 2010) reported finding organotins in house dust using a modification of the Sousa method
258 (Sousa, Laranjeiro et al. 2009).
259
260 Besides GC-MS, LC-MS has been used to analyze for the organotins. As
261 mentioned earlier because of the limitations of derivatization, there are methods that have
262 been developed that use LC/MS as a determinative method for organotins (and
263 organometallics) thus bypassing the need for derivatization and/or hydrolysis. Siu et
264 al. were the first to publish using atmospheric pressure chemical ionization (APCI)
265 ionspray tandem-MS in the selected reaction monitoring mode (SRM) for determining
266 organotin in sediment reference materials (Siu, Gardner et al. 1989). Another early
267 paper, published by Cullen et al. used a Kratos MS 80 RFA mass spectrometer equipped
268 with a Vestec Kratos thermospray interface to determine butyltins in marine samples
269 (Cullen, Eigendorf et al. 1990). Jones-Lepp et al. (1999) was one of the first papers to
270 demonstrate a rapid extraction and detection method for organotins in source waters
271 using LC-ESI-ion trap mass spectrometry (LC-ESI-ITMS) (Jones-Lepp, Varner et al.
272 1999). One inherent difficulty with their method was the instability of the electrospray
273 ionization process. Adding tropolone as a stabilizing reagent to the mobile phases
274 compensated for this difficulty. Because of the addition of tropolone, the ions detected
275 by the ion trap were the tropolonium adduct ions (Jones-Lepp, Varner et al. 1999). For

example, the spectrum for diphenyltin dichloride (mw 344 Da) is easily identified as mass m/z 395, produced from the loss of two chlorine atoms and the addition of the tropolonium ion, $(M\text{-}2Cl+C_7H_5O_2)^+$, figure 4. **<insert figure 4 of diphenyltin spectra>**

A recent publication shows a new technique of coupling on-line SPE to LC-ESI-MS and LC-atmospheric pressure chemical ionization (APCI)-MS for the speciation of organotins in waters (Sun, Chen et al. 2009). The direct coupling of SPE to the LC-MS allowed for removal of most matrix interferences and pre-concentration of tributyltin (TBT) and triphenyltin (TPT). The spectra appeared similar whether using ESI or APCI; for TBT the most prominent ion $(M\text{-}Cl)^+$ was detected at m/z 291.1 ($SnBu_3$), accompanied by fragment ions corresponding to the loss of one butene groups, $(TBT–C_4H_8)^+$, m/z 235.0, and two butene groups $(TBT–(C_4H_8)_2)^+$, m/z 179.0 (Sun, Chen et al. 2009). In Sano et al. (Sano, Takagi et al. 2010) they evaluated a hydrophilic interaction liquid chromatography (HILIC)–ESI-MS method. Their goal was to develop an improved and rapid liquid chromatography technique, HILIC, coupled to ESI-MS. The total chromatographic run times were under 15 minutes, with good separation between TPT and TBT. The ions detected under these conditions however were not what were expected. Instead of the molecular ions $(M)^+$, or $(M+H)^+$ being formed, the adduct ions of TBT and TPT, $[M+CH_3CN]^+$ at m/z 332 and m/z 392, respectively, were formed (Sano, Takagi et al. 2010). The formation of the acetonitrile adduct ions was due to the use of acetonitrile both in the extraction steps and in the mobile phase. With this method the LODs for TBT and TPT were 3 and 6 ng/L, respectively (Sano, Takagi et al. 2010).

Organoarsenics. While organotin derivatives are generally considered more toxic than

the inorganic forms of the element (Eskes, Honegger et al. 1999; Grun and Blumberg 2006; Moser, McGee et al. 2009), this is not the case for metalloid arsenic. Biomethylation of arsenic is considered to be a detoxification mechanism used by many organisms to counteract the effects of the more toxic inorganic forms of the element. Methylarsonic acid and dimethylarsinic acid, identified in many environmental matrices, were found to be less toxic than inorganic arsenic compounds (Kaise, Yamauchi et al. 1989). Trimethylarsine oxide was similar in acute toxicity to arsenobetaine, the most abundant and predominant arsenic species in many marine animals (Kaise, Yamauchi et al. 1989; Newcombe, Raab et al. 2010). In a recent study to understand the bioavailability of arsenobetaine in humans, researchers used a combination of LC-ICP-MS and LC-ESI-MS to confirm that the total arsenic seen by LC-ICP-MS was in fact due to just arsenobetaine. This was done by simultaneously obtaining LC-ICP-MS/ESI-MS data and monitoring the $(M+H)^+$ ion, m/z 179, of arsenobetaine (mw 178.06 Da). The $(M+H)^+$ is produced in the ESI source, alongside the element arsenic, m/z 75, in the ICP-MS, figure 5, confirming that the total arsenic found was due to arsenobetaine (Newcombe, Raab et al. 2010). Figure 5 shows the overlays of the LC-ICP-MS/ESI-MS chromatograms. <**insert figure from newcombe paper figure 5**>

Other organometallics. The toxicological and environmental impacts of many synthetic organometallic compounds that are used in medicine as anti-tumor agents or for other medicinal purposes (e.g., organoferrocenes, organoplatinum, organoboranes) have not been studied in detail (Cui, Ding et al. 2003; Hann, Stefánka et al. 2005; Lenz, Hann et al. 2005; Allard, Passirani et al. 2008; Johnson, Jörgens et al. 2008). Cui et al. (Cui, Ding

et al. 2003) report using a high-field asymmetric waveform ion mobility spectrometry (FAIMS) analyzer interfaced with ESI-ITMS to detect cisplatin (mw = 300.05 Da, $Cl_2H_6N_2Pt$) in solutions. They make use of the filtering (mass) ability that FAIMS offers to show dramatic improvements in detection of cisplatin by significantly reducing the background "noise" by 30-fold (Cui, Ding et al. 2003). This technique holds the potential to cross-over into the environmental field.

Pharmaceuticals and other drugs. In recent years it has been clearly demonstrated that pharmaceuticals, both human-use and veterinary, can find their way into the natural environment after excretion or disposal by end-users (Daughton and Ternes 1999; Daughton and Jones-Lepp 2001; Ankley, Brooks et al. 2007; Kemper 2008; Kümmerer 2010). Acceptable, reproducible, and sensitive analytical chemistry techniques are necessary to better quantify and support environmental and human-health risk assessments from pharmaceuticals detected in the environment. The majority of the detection techniques to identify very low levels (ppb and lower) of pharmaceuticals in complex environmental matrices are mass spectrometry based (Daughton 2001; Petrovic, Hernando et al. 2005; Hao, Clement et al. 2007; Wang 2009; Richardson 2010).

Initially GC/MS was the approach that had been used for detecting non-polar pharmaceuticals, polar pharmaceuticals (with derivatization) and steroids and hormones in environmental matrices. The first report of drugs, drug metabolites, and steroids detected in the environment was by Garrison et al. in 1976 (Garrison, Pope et al. 1976). The ECs and other "traditional" compounds (e.g., aromatic hydrocarbons, phthalates,

chlorinated alkanes, etc.) were extracted using a liquid-liquid extraction (LLE) procedure followed by methylation, and GC/MS detection (Garrison, Pope et al. 1976). Moeder et al. (2000) developed a solid-phase microextraction (SPME) extraction method with subsequent derivatization, for detecting ibuprofen, clofibric acid, caffeine, paracetamol, phenazone, carbamazepine, gemfibrozil, naproxen, indomethacine, norethisteron, propranolol, and metaprolol in water, using GC/MS/MS (Moeder, Schrader et al. 2000). The authors describe adding 100 µL of (BSTFA) to the 500 µL SPME extract, heating for 1-hr at 40^{o}C, evaporating to 250 µL, then injecting 1 µL into a GC/MS for analysis (Moeder, Schrader et al. 2000).

However, many pharmaceuticals and hormones are polar, thermally instable, hydrophobic, and have low volatility, making them ideal candidates for LC/MS. Therefore, today almost all methods for detecting polar pharmaceuticals in the environment are LC-MS and LC-MS/MS based techniques (Petrovic, Hernando et al. 2005; Kosjek, Heath et al. 2007). As mentioned earlier, Moeder et al. (2000) developed a SPME extraction method, with derivatization, for detecting several pharmaceuticals in water, so that the extract was suitable for GC/MS/MS analysis (Moeder, Schrader et al. 2000). Farré et al. (2001) developed a SPE extraction method, minus derivatization, for the same analytes from water (Farré, Ferrer et al. 2001). They analyzed the extracts directly by negative ionization LC-ESI/MS, and found that the LC/MS method was an improvement over the GC/MS method since the derivatization step was avoided (Farré, Ferrer et al. 2001).

Conley et al. (2008) describe a rapid detection method using ultra performance liquid chromatography (UPLC) coupled to a ESI-QqQ for a large number of common pharmaceutical ECs (Conley, Symes et al. 2008). The use of UPLC allowed for the detection of 13 different pharmaceuticals, and one metabolite, in less than 5 minute chromatographic runs. With UPLC coupled to ESI-QqQ their ability to correctly identify these analytes was enhanced into the low ng/L (ppt) range (Conley, Symes et al. 2008). One important issue that Conley et al. (2008) bring up is that of matrix effects when using LC-ESI-MS techniques (Conley, Symes et al. 2008), and providing an equation for determining whether the detection of an analyte is matrix enhanced or suppressed. Other researchers have noticed these phenomena of matrix suppression or enhancement when using LC-ESI-MS. For example, during the analysis of sulfonamides and tetracyclines with LC-ESI-iontrap MS/MS, Yang et al. (2004) found ionization suppression of tetracyclines in wastewater to be significant, while no suppression or enhancement of the signal for sulfonamides was observed. The matrix suppression of tetracyclines was alleviated by using an internal standard (Yang, Cha et al. 2004). Matrix interferences in Swedish hospital wastewater was also found to be negligible for sulfamethoxazole by Lindberg et al. (2004), while ciprofloxacin was found to be highly susceptible to matrix ionization suppression (Lindberg, Jarnheimer et al. 2004).

A recent publication by Loos et al. (2010) demonstrates the flexibility and potency of LC-MS/MS as a screening technique (Loos, Locoro et al. 2010). They screened for 34 ECs (e.g., pharmaceuticals, pesticides, EDCs, PFOA/PFOS, etc.) using LC-ESI-atmospheric pressure ionization-QqQ (LC-ESI-API-QqQ). They had to use both

391 the positive and negative ionization modes in order to capture all the chemical classes
392 represented by the 34 ECs (Loos, Locoro et al. 2010). Another recent development in
393 unique environmental screening mass spectrometry tools is the liquid chromatograph-
394 hybrid linear ion trap-fourier transform-orbitrap mass spectrometry (LC–LTQ FT-OT)
395 (Hu, Noll et al. 2005). The OT combines the high resolution and mass accuracy
396 acquisition capability to capture MS^n spectra (Hu, Noll et al. 2005). Environmental
397 samples are complex, such that not only are targeted analytes present, but so are
398 numerous unknown ECs. In Hogenboom et al. (2009) the use of LC–LTQ FT-OT
399 allowed for a two pronged approach to identify targeted analytes, as well as the
400 identification of several unknown ECs that were present in a groundwater sample
401 (Hogenboom, van Leerdam et al. 2009). First Hogenboom et al., made full-scan accurate
402 mass measurements of the unknown ECs and then compared those measurements with
403 theoretical exact masses of known ECs. When they couldn't find a complete match they
404 modeled elemental compositions of the unknown ECs. MS^n experiments were performed
405 to obtain fragment ions in the LTQ, and they used the OT portion of the mass
406 spectrometer to generate accurate mass measurements on the fragment ions. Using what
407 they termed a "fragmentation tree" they were able to link the accurate mass fragment ions
408 to the accurate mass precursor ions generated during the full-scan mode (Hogenboom,
409 van Leerdam et al. 2009). Figure 6 demonstrates the ability of this technique to
410 accurately identify a previously unknown EC from a complex environmental matrix.
411 **<insert fig 6 Hogenboom paper>** In figure 6, shown in the top half is the full-scan
412 accurate mass spectrum of initially an unknown EC in the groundwater extract.
413 However, after applying their "fragmentation tree" formula they were able to determine

414 that the unknown EC is metolachlor oxalinic acid. The bottom half of the figure shows
415 the spectrum from a standard solution of metolachlor oxalinic acid, proving their
416 determination was correct (Hogenboom, van Leerdam et al. 2009).
417
418 **Nanomaterials.** To be able to be fully informed in risk assessments regarding both
419 human health and environmental exposure to nanoparticles the need exists for the
420 technology and methods to detect nanoparticles in environmental matrices. Engineered
421 nanomaterials, especially nanosilver, are already heavily used by consumers in such
422 products as cosmetics, socks, underwear, washing machines, etc., thereby increasing the
423 chance of the release of these nanomaterials into the aquatic environment through
424 wastewater treatment plant effluents or the accidental releases of raw sewage (Geranio,
425 Heuberger et al. 2009; Howard 2010; Weinberg, Galyean et al. 2011). Since little data
426 currently exists in the literature regarding ecotoxicological effects from these materials,
427 the first steps would be to (1) determine if engineered nanomaterials are present in the
428 environment, and (2) in what concentrations, bringing with it analytical challenges.
429 Analytical methods need to be able to differentiate between naturally occurring and
430 engineered nanoparticles, size differentiate and separate the engineered nanoparticles
431 from everything else present in complex environmental matrices. A few researchers have
432 used the technique of LC/MS towards understanding the fate and transport of the
433 fullerene class of nanomaterials.
434
435 Isaacson et al. (2007) demonstrate an LC-ESI-MS detection method for several
436 fullerenes, showing a clear chromatographic separation of each fullerene, i.e., C_{60}, $^{13}C_{60}$,

437 C_{70}, C_{82}, C_{88}, C_{98}, figure 7. **<insert fig7 from Isaacson article>** Using the negative
438 ionization mode the most abundant ions formed under ESI-MS conditions were the
439 molecular ions [M ·]. They were able to successfully separate and quantitate each of the
440 fullerenes at low, environmentally relevant concentrations. For example, the LOD for
441 C_{60}, as defined by the concentration that gave a signal-to-noise ratio of 3:1, was 0.0004
442 µg/L (Isaacson, Usenko et al. 2007). Chen et al. (2008) developed a SPE method to
443 concentrate fullerene nanomaterials from natural waters. The SPE technique eliminated
444 background interferences such that low environmentally relevant levels of fullerenes
445 could be detected using an atmospheric pressure chemical ionization source (APCI) with
446 LC-MS, LC-APCI-MS (Chen, Westerhoff et al. 2008). Using SPE with LC-APCI-MS
447 allowed them to detect C_{60} as the negative-ion, m/z 720 [M ·]. Based upon the response
448 of the signal from m/z of 720 [M ·], C_{60} appeared to be detected as a single nanoparticle
449 under LC-APCI-MS conditions (Chen, Westerhoff et al. 2008). Isaacson and Bouchard
450 (2010) have recently coupled asymmetric flow field flow fractionation (AF4) to a
451 dynamic light scattering detector in flow through mode, and processed the fractionated
452 sample using an atmospheric pressure photoionization source coupled to LC-MS, LC-
453 APPI-MS (Isaacson and Bouchard 2010). This technique allowed for unambiguous
454 determinations of C_{60} in each of the size fractions collected from AF4.
455
456 At this time the current literature does not indicate the ability to detect
457 nanometallics (e.g., nanosilver, nanogold, nanoiron) except through the use of a few non-
458 specific detectors, e.g., ICP-MS, UV-vis, fluorescence (Howard 2010; Weinberg,

Galyean et al. 2011). While these non-specific detectors can give total metal content, for the most part they lack the ability to differentiate between naturally occurring and engineered nanoparticles, and specificity in particle sizing and counting. There is a need for the ability to couple non-specific detectors with AF4, or other size exclusion techniques and to improve upon their capabilities in particle counting and reduction of background interferences (Howard 2010).

Oil spill dispersants. Dispersants are detergent-like chemicals comprised of surfactants (surface-active agents) dissolved in one or more solvents. They are designed to be sprayed onto oil spills to remove oil from the sea surface and disperse it below the surface and into the water column. The application of dispersants is intended to accelerate the degradation of the oil's chemical constituents (e.g., hydrocarbons, polynuclear aromatic hydrocarbons) by dilution and natural bacterial processes, thereby reducing or eliminating the environmental impact of the oil (Lessard and DeMarco 2000).

As mentioned early in the chapter, in response to the oil spill of the Deepwater Horizon Incident (DWHI) nearly 2 million gallons of dispersants (i.e., Corexit 9500 and Corexit 9527) had been deployed (http://www.restorethegulf.gov/release /2010/12/01/operations-and-ongoing-response-december-1-2010) through aerial spraying, and underwater deployment. It was through the use of mass spectrometry that researchers at the USEPA National Exposure Research Laboratory-Las Vegas, Nevada (NERL-Las Vegas) were able to decipher the chemical contents of the dispersants used on DWHI. Several researchers, using a variety of mass spectrometric techniques,

482 determined the individual chemical constituents of the various fractions (e.g., volatiles,
483 semi-volatiles, and non-volatile organics) of the dispersants. Serial dilutions of the two
484 dispersants (i.e., Corexit 9500 and Corexit 9527) were made up in: water for determining
485 the volatile organics (VOAs); hexane for the semi-volatiles (semi-VOA); and methanol
486 for the non-volatile fractions. For the VOA fraction the diluted dispersants were
487 analyzed by vacuum distillation-GC-MS (VD-GC-MS); this technique is suitable for
488 those VOAs that are primarily in the boiling point range between 180 and 240^{o} C (Hiatt
489 1995), http://www.epa.gov/epawaste/hazard/testmethods/sw846/new_meth.htm
490 - 8261A). The results of both dispersants (9500 and 9527) showed that the VOA fraction
491 consisted mainly of a low-boiling solvent(s) (the solvents are for helping in the aerial
492 dispersing), lighter weight alkyl aromatic hydrocarbons, simple hydrocarbons, 1,4-
493 dioxane, and naphthalene. The semi-VOA fraction of Corexit 9500 was analyzed by GC-
494 MS; one main component was determined, bis (2-ethylhexyl) fumarate (CAS # 141-02-
495 6); this compound is the industrial precursor to sodium dioctyl sulfosuccinate (DOSS)
496 [DOSS was reported as a constituent in Corexit 9500 by the National Academy of
497 Sciences (NAS) (Committee on Understanding Oil Spill Dispersants: Efficacy and
498 Effects 2005)], as well as a smaller peak that was identified as an isomer of bis (2-
499 ethylhexyl) fumarate. The non-volatile fraction of Corexit 9500 was analyzed by LC-
500 ESI-ITMS and direct analysis in real-time time-of-flight mass spectrometry (DART-
501 TOFMS), two complementary LC-MS techniques. DART-TOFMS is a rapid screening
502 technique that gives accurate mass of those unknown dispersant compounds that are
503 ionizable (Grange and Sovocool 2008), while LC-ESI-ITMS allowed for separation, and
504 detection using MS/MS capability to determine the unknown constituents in the

dispersant. Using these two techniques it was determined that the major non-volatiles present, in the positive ionization mode, were nonionic surfactants (e.g., ethoxylated sorbitan mono- and trioleates) [as reported by NAS (Committee on Understanding Oil Spill Dispersants: Efficacy and Effects 2005)], dipropylene glycol n-butyl ether, and a minor amount of nonylphenol ethoxylate. In the negative ionization mode, using LC-ESI-triple quadrupole mass spectrometry (LC-ESI-QqQ), the presence of DOSS [as reported by the National Academy of Sciences (NAS) (Committee on Understanding Oil Spill Dispersants: Efficacy and Effects 2005)] was confirmed.

Mass spectrometry was also used to provide quality assurance/quality control (QA/QC) support to toxicological testing of dispersants (Judson, Martin et al. 2010). Spiked well-plates (to be used for toxicological testing), **<insert figure 8 of picture of well-plate>** and mixtures of sea water/oil/dispersants, were analyzed as part of QA/QC measures using the same multiple mass spectrometry techniques that were used to determine the dispersant constituents. The importance of using mass spectrometry was shown during the cross-checking of the well-plates. The first example is demonstrated in the mass spectra and chromatogram of a standard of dispersant A, figure 9 (a). **<insert figure 9 of two dispersant spectra (a) and (b)>** Shown are a series of ions that are attributable to multiple surfactants (e.g., oxylated sorbitan oleates). One series of oxylated oleates is: m/z 476.3, m/z 520.3, m/z 564.3, m/z 608.4. Each series of ions are separated by mass m/z 44, which is attributable to ($-CH_2CH_2O-$), indicating an ethoxylated species of sorbitan oleates. Present in the same spectrum is another, lesser, underlying series of ethoxylated ions at m/z 503.3, m/z 547.3, m/z 591.3. Also detected

in this non-polar fraction of dispersant A is a large peak at mass m/z 163.2, $(M+H)^+$, and a smaller peak at m/z 185.2 $(M+Na)^+$, the sodium adduct ion. Using DART-TOFMS, and specialty software, the m/z 163.2 $(M+H)^+$ was determined to be attributable to either, m/z 162.13, 2-Propanol, 1-(2-ethoxypropoxy)-, or m/z 162.13, 2-(2-Butoxyethoxy) ethanol. However, it would have been necessary to obtain standards of each of these compounds to further accurately clarify which analyte was actually detected. The second example is demonstrated in figure 9 (b). Shown in this figure is a mass spectra and chromatogram of a well-plate that was supposed to contain only dispersant A. However, also seen in the same mass spectra and chromatogram, as a minor contaminant, are the ions attributable to dispersant G, m/z 191 and m/z 213. These two instances, determining unknowns and cross-checking for accuracy demonstrate the power of mass spectrometry techniques so magnificently.

Conclusions

In this chapter we have tried to cover a broad range of mass spectrometric analytical techniques applicable to extracting and detecting ECs from complex environment samples. Many of the techniques discussed are new technologies built upon dependable older mass spectrometric techniques. What we have not covered are the myriad of ways to sample and extract ECs from complex environmental samples. The reader is encouraged to go to the literature to learn more about these subjects (Jones-Lepp, Alvarez et al. 2009; Richardson 2010). Most of the mass spectrometric techniques that are published regarding the detection of ECs in the literature also explain the sampling and extraction techniques that were used for those particular classes of ECs.

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Tables

Table 1. Emerging Contaminant Subcategories, Classifications and Available Analytical Methods

873 **Table 1. Emerging Contaminant Subcategories, Classifications and Available Analytical Methods[1]**
874

Emerging Contaminant Subcategory	Compounds included in Emerging Contaminant Subcategory
Pharmaceuticals/Illicit drugs	- Life style drugs - Antidepressants - Hormone replacements and ovulation inhibitors - some antibacterials and antimicrobials - Prescription and over the counter human and veterinary medications
Personal Care Products	- Musks, some antibacterials and antimicrobials - Chemicals found in hygiene products - High usage in everyday household items
Steroids and Hormones	- anabolic agents - hormone replacements, - sex Hormones - ovulation inhibitors - Phytosterols and various other sterols - Moderate to high lifestyle and medicinal uses, some illicit uses
Surfactants	- Nonyl phenols - alkyl phenols - linear alkyl ethoxylates - ethoxylated sorbitan monoleates - ethoxylated sorbitan trioleates
Organometallics	- Medicinals - Fungicides - Molluscides - dyes
Nanomaterials	Antimicrobial/antivirals, makeup, sunscreen agents, imaging agents

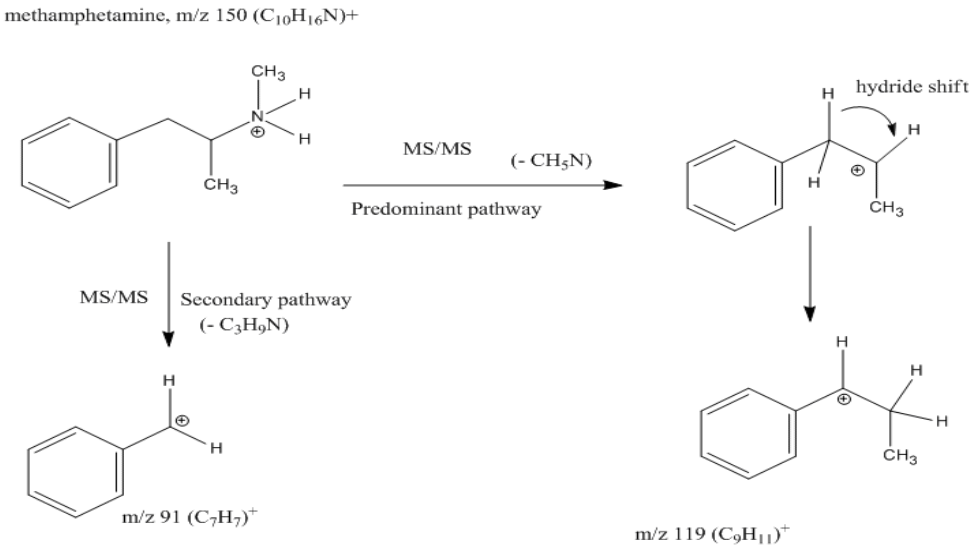
875 [1] The above table is not intended to be exhaustive. Original design of table courtesy Dr. Brian Englert, Greenguard
876 Environmental Institute, Marietta, Georgia USA

Figures

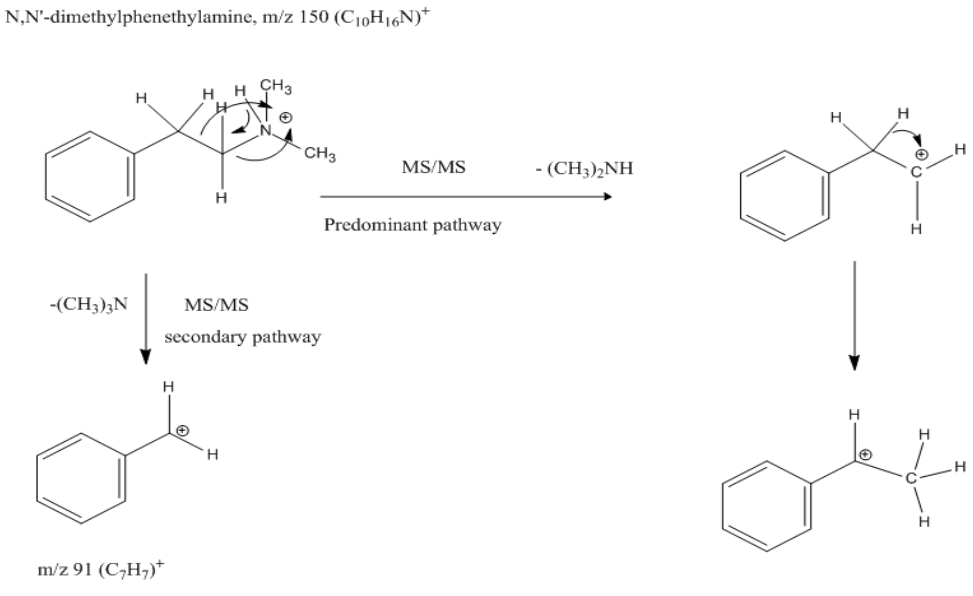
1. Precursor to product pathways: (a) methamphetamine and (b) DMPEA Jones-lepp
2. Precursor to product pathways: (a) MDMA and (b) caffeine Jones-lepp
3. Mass spectra of four organotin species: tetraethyltin, tributyltin, diphenyltin, and triphenyltin obtained from derivatization and GC-MS analysis. Segovia-Martínez
4. Mass spectra of diphenyltin obtained from LC-ESI-ITMS Jones-lepp
5. Comparison of LC-ICP-MS/ESI-MS spectra of mass m/z 75, total arsenic, to mass m/z 179 arsenobetaine. Newcombe
6. Full-scan accurate mass spectrum (negative-ion mode) of metolachlor oxalinic acid (metolachlor OA) detected in a groundwater sample (top) and a standard solution (bottom). Hogenboom et al. 2009
7. LC-ESI-MS chromatogram of several fullerenes. Isaacson 2007
8. Toxicity testing well-plate. Jones-lepp
9. Mass spectra and chromatograms of Dispersant A and well-plates
    a. Standard of Dispersant A
    b. Well-plate Dispersant A

Figure 1. Precursor to product pathways: (a) methamphetamine and (b) DMPEA

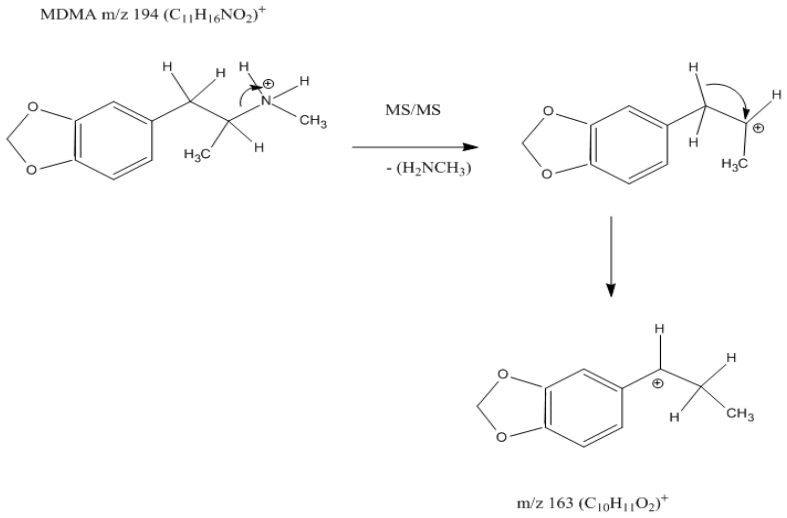
(a)



(b)



907 Figure 2. Precursor to product pathways: (a) MDMA and (b) caffeine
908
909 (a)



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914 (b)

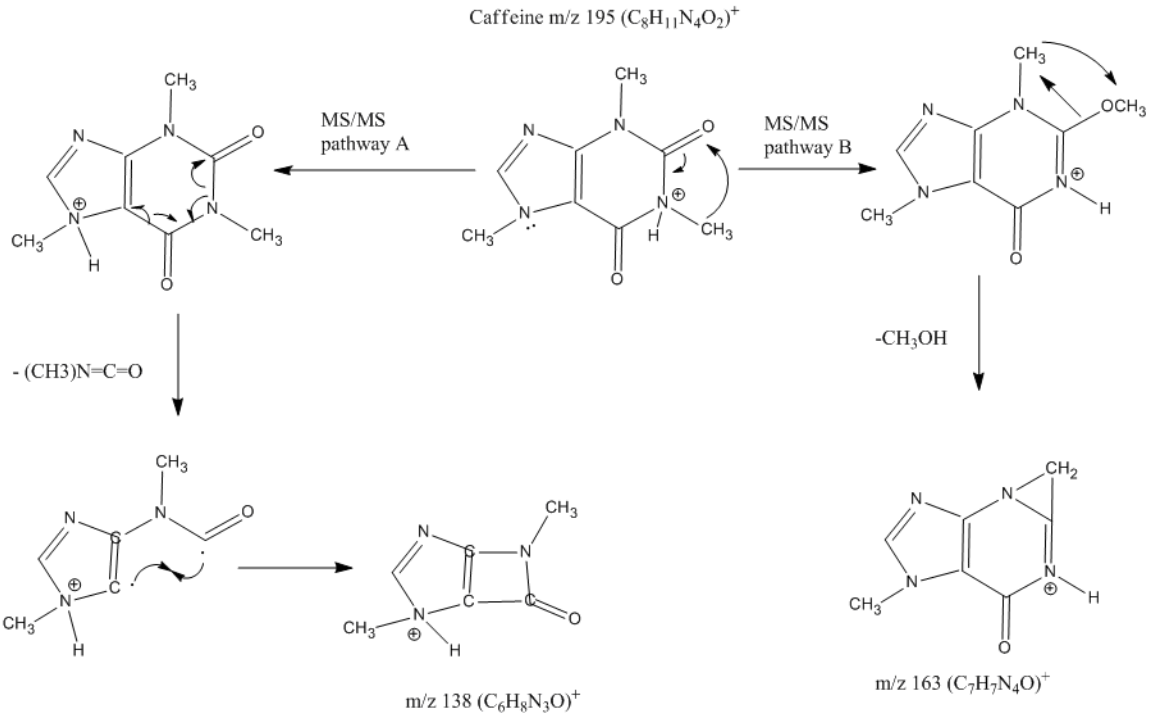

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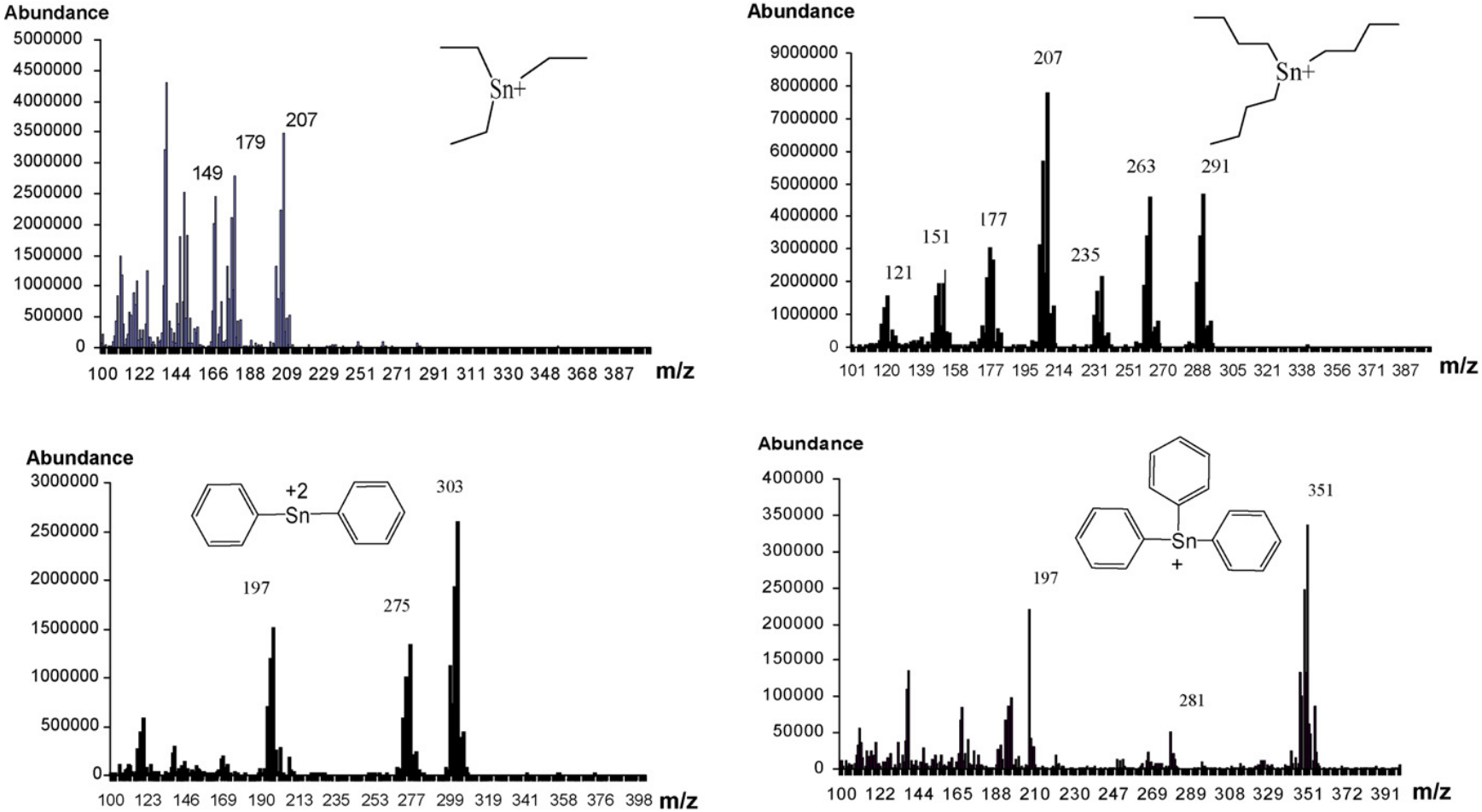
Figure 3. Mass spectra of four organotin species: triethyltin, tributyltin, diphenyltin, and triphenyltin obtained from derivatization and GC-MS analysis



Reprinted from Talanta, 80, L. Segovia-Martínez, A. Bouzas-Blanco, P. Campíns-Falcó, A. Seco-Torrecillas, Improving detection limits for organotin compounds in several matrix water samples by derivatization-headspace-solid-phase microextraction and GC–MS figure 4, pgs. 1888–1893, 2010, with permission from Elsevier

Figure 4. Mass spectra of diphenyltin obtained from LC-ESI-ITMS

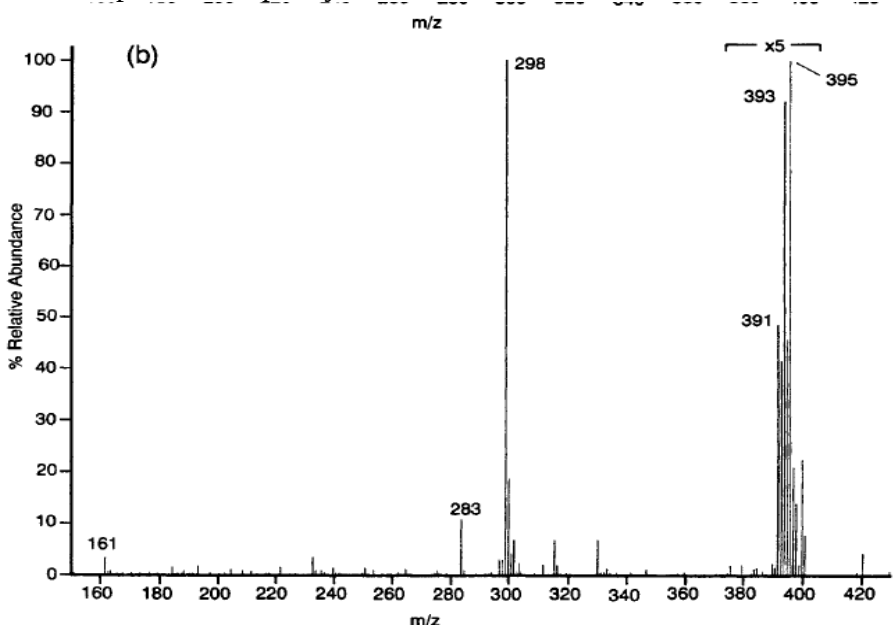


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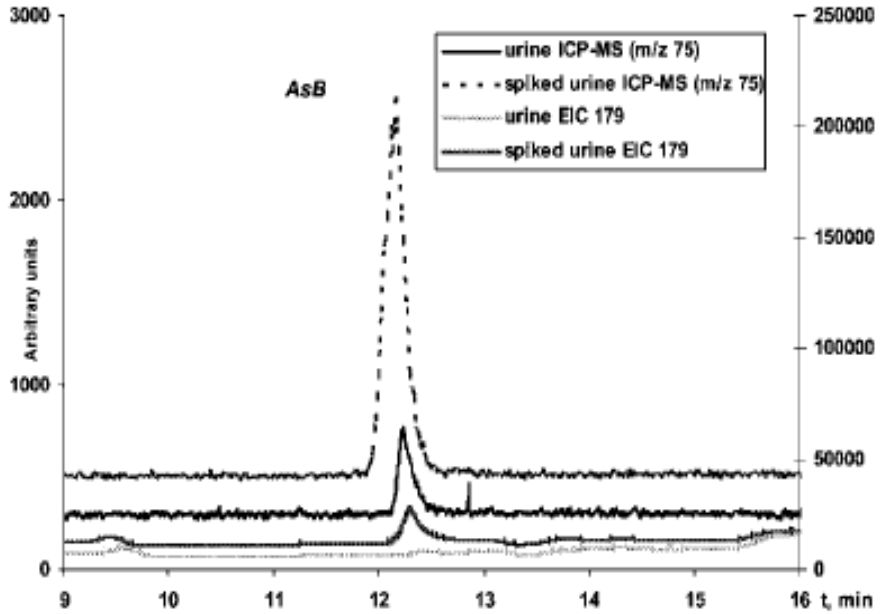
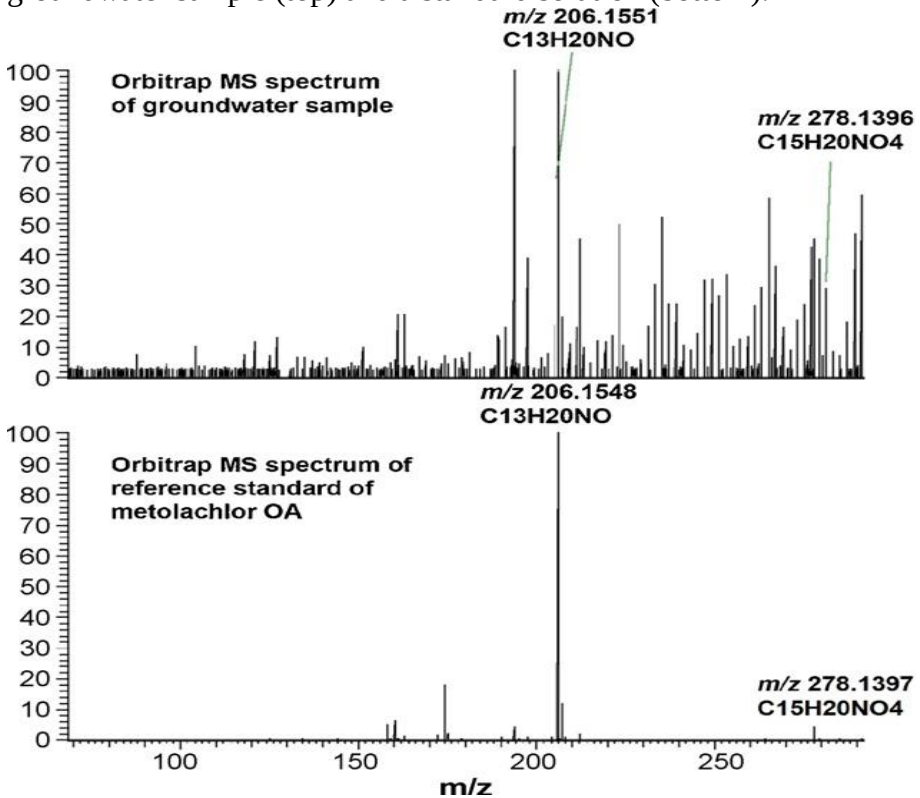
Figure 5. Comparison of LC-ICP-MS/ESI-MS spectra of mass m/z 75, total arsenic, to mass m/z 179 arsenobetaine.



Reprinted from J. of Environmental Monitoring, figure 2 from Newcombe et al. "Accumulation or production of arsenobetaine in humans," 2010, 12, 832-837, with permission from Royal Society of Chemistry.

Figure 6. Full-scan accurate mass spectrum (negative-ion mode) of metolachlor oxalinic acid (metolachlor OA) detected in a groundwater sample (top) and a standard solution (bottom).



Reprinted from J. of Chromatogr. A, 1216 (3), figure 3 from Hogenboom et al. "Accurate mass screening and identification of emerging contaminants in environmental samples by liquid chromatography-hybrid linear ion trap orbitrap mass spectrometry." 510-519, (2009) with permission from Elsevier.

Figure 7. LC-ESI-MS chromatogram of several fullerenes.

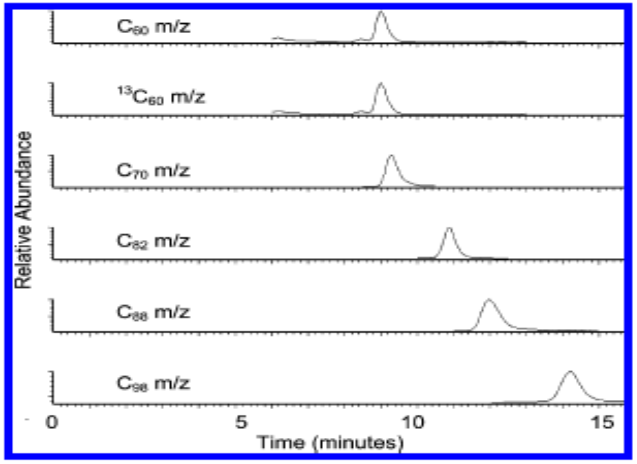
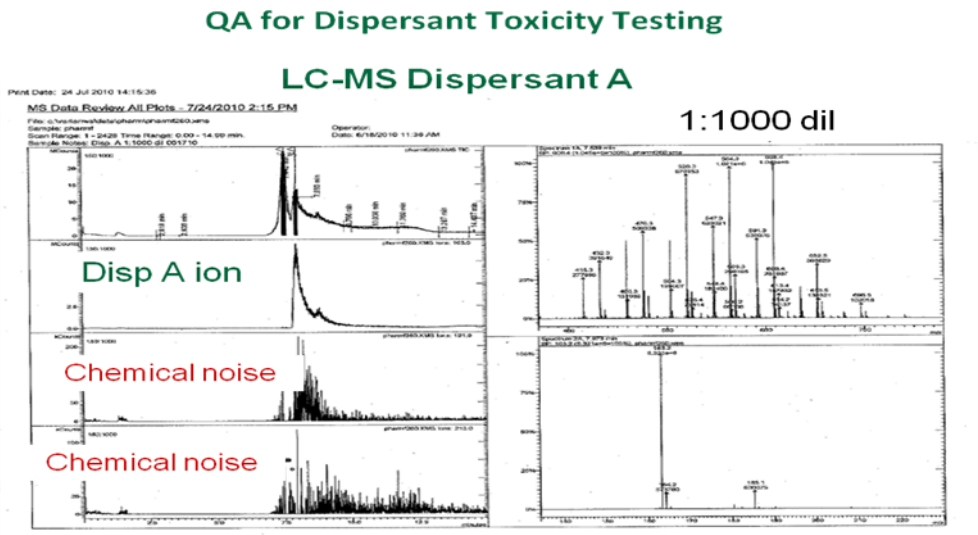


Figure 1. Selected LC/ESI-MS chromatograms in methanol/toluene (80:20) unless otherwise noted; including C_{60} (2 μg/L in zebrafish homogenate matrix), $^{13}C_{60}$ (10 μg/L in zebrafish homogenate matrix), C_{70} (10 μg/L), C_{82} (3.4 μg/L), C_{88} (2.5 μg/L), and C_{98} (0.4 μg/L). Additional fullerenes in higher-order mixture not shown.

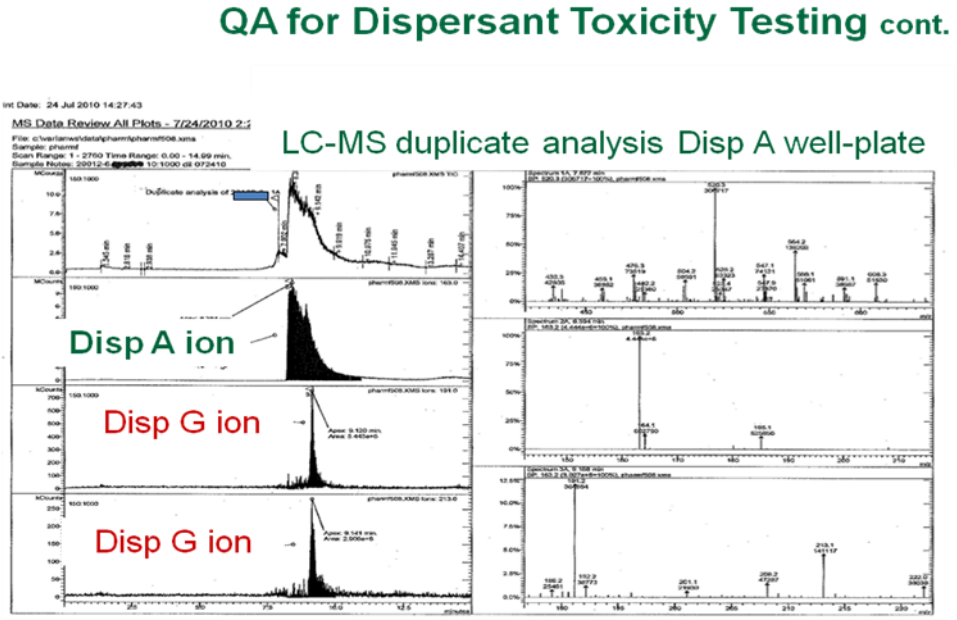
Reprinted in part with permission from figure 1, Isaacson, C. W., C. Y. Usenko, et al. (2007). "Quantification of Fullerenes by LC/ESI-MS and Its Application to in Vivo Toxicity Assays." Analytical Chemistry 79(23): 9091-9097. Copyright 2007 American Chemical Society.

Figure 8. Toxicity testing well-plate

Figure 9. Mass spectra and chromatograms of Dispersant A and well-plates (a) Standard of Dispersant A; (b) Well-plate Dispersant A



(a)



(b)