On the frontier: Analytical chemistry and the occurrence of illicit drugs in surface waters in the USA.

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Introduction

While environmental scientists focused on industrial and agricultural pollutants (e.g. PCBs, volatile organics, dioxins, benzene, DDT) in the 1970's and 1980's, overlooked was the subtle connection between personal human activities, such as drug consumption, and the subsequent release of anthropogenic drugs and drug metabolites into the natural environment. There was evidence of this possible connection nearly 30 years ago when Garrison et al. (1976) reported the detection of clofibric acid (the bioactive metabolite from a series of serum triglyceride-lowering drugs) in a groundwater reservoir that had been recharged with treated wastewater.(Garrison et al. 1976) A year later Hignite and Azarnoff (1977) reported finding aspirin, caffeine, and nicotine in wastewater effluent, and then Watts et al. (1983) reported the presence of three pharmaceuticals (erythromycin, tetracycline, and theophylline), bisphenol A and other suspected endocrine disrupting compounds (EDCs) in a river water sample.(Hignite and Azarnoff, 1977; Watts et al. 1983) Following those three journal articles there, nothing was published for nearly a decade regarding the drug-human-environmental connection. Renewed interest in the subject was reported by Daughton and Ternes's seminal and authoritative work published in 1999.(Daughton and Ternes, 1999) Since the 1999 publication of Daughton and Ternes's, the number of publications from the scientific community regarding the human drug consumption and environmental interaction have increased from two publications in the1980's to currently over 300 scientific publications per year. Most of these publications report methods for the detection of common

pharmaceuticals and over-the-counter (OTCs) drugs. However, very few publications have dealt with the occurrence, transport, and fate of illicit drugs in the environment.

In the United States (US), Snyder et al. (2001) reported the presence of hydrocodone, codeine, and diazepam (valium), in a stream entering into Lake Mead, Nevada.(Snyder et al., 2001) While these drugs are not considered illicit substances, they are considered controlled substances, compounds that the Drug and Enforcement Agency (DEA) lists as schedule III and IV drugs, as substances for potential abuse. (DEA, http://www.usdoj.gov/dea/pubs/ abuse/1-csa.htm) Then for the first time the presence of an illicit substance, methamphetamine, was reported by Khan and Ongerth, in wastewater effluent from a large US city in California and announced publicly at the 2003 National Ground Water conference.(Khan and Ongerth, 2003) Jones-Lepp et al. (2004) reported for the first time in the peer-reviewed literature the detection of two illicit drugs, methamphetamine and methylenedioxy-methamphetamine (MDMA, Ecstasy), collected from wastewater treatment plant (WWTP) effluent streams in Nevada and South Carolina, US.(Jones-Lepp et al., 2004)

In the US, there are the following possible sources of release of illicit drugs into US waterways. The largest possible contributor of illicit drugs would be from consumer consumption, and subsequent excretion into the municipal sewer systems and transport through the WWTP process into streams, lakes, rivers, or wetlands.(Jones-Lepp et al., 2004; Chiaia et al., 2008; Loganathan et al., 2009; Bartelt-Hunt et al., 2009) A smaller

contribution could be from consumer consumption and subsequent excretion into septic systems, or other non-seweraged systems (e.g., boat privies, outhouses), and then leakage from the septics into surrounding source waters, creeks, bays, and wetlands.(Jones-Lepp 2006) Another possible source of illicit drugs can be from runoff from biosolids that have been applied as soil amendments to crops, municipal parkways, or during forest restoration.(Kaleta et al., 2006; Kinney et al., 2006; Jones-Lepp and Stevens, 2007; Edwards et al., 2009) A likely source of illicit drugs could be from clandestine drug laboratories. For example, during the illegal manufacturing of methamphetamine well over 50 hazardous chemicals are either used, or produced, as methamphetamine by-products.(US EPA, 2008) All of these hazardous compounds, including methamphetamine, have the potential to enter the environment through improper disposal into the city sewer or individual septic systems, or via shallow drainage ditches directly onto surrounding soils (commonly used in remote methamphetamine operations), and through burn or burial pits.(US EPA 2008)

Another aspect of environmental monitoring of illicit drugs is socioeconomic. Daughton in 2001 was the first researcher to comment on developing an environmental monitoring program for the use of illicit substances.(Daughton, 2001) Daughton proposed using sewerage monitoring to provide data on the daily influxes of drugs from a community and applying this data to obtain a realistic perspective on the overall magnitude and extent of community illicit drug use. Using Daughton's premise, two epidemiology studies have been completed in Europe (Italy, Spain) (Zuccato et al., 2005;

Postigo et al., 2009). Recently, in 2009, the first epidemiologic study, using Daughton's premise, was completed in the US and published.(Banta-Green et al., 2009)

Besides environmental monitoring data and as important is the lack of data regarding the ecotoxicity of the pharmaceuticals and illicit drugs. The missing ecotoxicity data makes estimations of predicted no-effect concentrations (PNEC), and hazard and risk assessments almost impossible, or at worse, a "best guess" scenario. Some researchers try to derive risk assessment data from the use of models that use quantitative structure-activity relationships (QSARs) and other measurements.

In the absence of empirical environmental data, one might be tempted to use such models as EPA's Ecological Structure Activity Relationships (ECOSAR) program, which is insufficiently accurate to actually predict ecotoxicity.(Fent el al., 2006) For example, the collapse of the vulture populations in India due to exposure to minimal (sub-therapeutic doses) amounts of diclofenac would never have been predicted with modeling.(Oaks et al., 2004) Even more critical is generating risk assessments for those organisms that live in the aquatic environment. Even though acute toxicity may not be a high risk, chronic exposure to sub-lethal doses may alter an aquatic organisms feeding and mating behaviors. Brown et al. (2007) demonstrated the deficiencies of trying to model bioconcentration factors (BCFs) versus actual field measurements in fish plasma.(Brown et al., 2007) There were extreme differences for some of the compounds measured, and Brown points out the importance of using real-life exposures to test

100 theoretical models at an early stage in model development.(Brown et al., 2007)
101
102 Ecotoxicological consequences of illicit drugs being deposited into environmental
103 matrices, particularly water, have not been closely examined. Therefore, it can only be
104 surmised that these substances may have the potential to adversely affect biota that are
105 continuously exposed to them, even at very low levels. The potential for chronic effects
106 on human health is also unknown, and of increasing concern due to the multi-use
107 (continuously recycled in a closed-loop) character of water, as in densely populated arid
108 areas. The focus of this chapter will be on the state-of-the-art in sampling, extraction and
109 analysis of illicit drugs in the waterways of the US. However, since much of the work
110 with illicit drugs has been performed outside the US, some of that data will also be given
111 as examples. Better characterization of illicit drugs in the environment forms the
112 foundation of improved risk assessments and sound science-based environmental policy.
113
114 **Physical-Chemical Properties of Illicit Drugs**
115
116 The persistence of illicit drugs or any chemical in an aquatic ecosystem depends on its
117 physical-chemical and ecosystem-specific properties. Among these are concentration of
118 dissolved/suspended organic matter, solubility, microbial population, etc. (Baughman et
119 al., 1978; Loganathan and Kannan, 1994) Persistence of methamphetamine, MDMA and
120 related compounds in aquatic systems are a function of physical (e.g., volatilization from,
121 and adsorption to, suspended solids and sediment), chemical (hydrolysis, photolysis) and

biological removal (microbial degradation, uptake) mechanisms in addition to flow and other water characteristics.(Loganathan et al., 2001) Considering the chemical makeup of illicit drugs, the volatilization of these compounds from natural water and sediment mixture is minimal, due to adsorption onto suspended solids or sediment.(Loganathan et al., 2009) Very limited information is available on the half-lives of illicit drugs in water, sediment, and biota. For example, cocaine hydrochloride's water solubility is 0.17 g/100 mL, whereas its solubility in ether is 28.6 mg/100 mL, and the boiling point is about 188°C, these characteristics indicate that it is compatible with organic matter and will adsorb onto solid materials.(Claustre and Bresch-Rieu, 1999) Photolysis of small molecules, such as methamphetamine and MDMA, may be possible in clear surface waters; however, there photolysis rates for these chemicals are not available.

The *p*Ka, along with log D_{OW} (the pH-dependent *n*-octanol-water distribution ratio), can provide strong evidence of whether compounds will be in an ionized state and their hydrophobicity.(Wells, 2006) These two physical chemical properties can help determine whether they will be retained in water, biosolids, sediment and/or biological medium. For example, the *p*Ka's and log D_{OW} of methamphetamine, MDMA, cocaine, all weak bases, were 9.9 pKa/-0.23 log D_{OW}, 10.38 pKa/-1.11 log D_{OW}, and 8.6 pKa/1.83 log $D_{OW,}$ respectively.(pKa: methamphetamine, Logan, 2002; MDMA, Tsujikawa et al., 2009; and cocaine, Domènech et al., 2009 ;log D_{OW} was calculated using SPARC program, at pH 7, http://ibmlc2.chem.uga.edu/sparc/index.cfm). Although all three compounds have been detected in the water column, the log D_{OW}'s would suggest that

only methamphetamine and MDMA will make it through the WWTP process and into the water column, while cocaine may be more likely to partition to the solids.(Logan, 2002; Garrett et al., 1991; Jones, 1998) Structures and select physicochemical properties of a few common illicit drugs are given in Figure 1 and Table 1.

There are four efficiency studies available that look at the removal of illicit drugs from WWTPs.(van Nuijs et al. 2009; Huerta-Fontela et al. 2008a; Castiglioni et al. 2006a; Loganathan et al. 2009) However, we can use the data from van Nuijs et al. (2009) and Loganathan et al. (2009) to illustrate the importance of using log D_{OW}, in conjunction with pKa, to predict removal and partitioning. If we consider the log D_{OW} of cocaine and methamphetamine, 8.6 pKa/1.83 log D_{OW} and 9.9 pKa/-0.23 log D_{OW}, respectively, one would predict that cocaine (log $D_{OW} > 1$) would be removed from wastewater more efficiently than methamphetamine (log $D_{OW} < 1$). And indeed van Nuijs et al. (2009) showed that cocaine is nearly 100% removed by those WWTPs using conventional activated sludge (CAS) treatment, and Loganathan et al. (2009) calculated the removal efficiency of methamphetamine at 55% at another WWTP that also uses CAS.(van Nuijs et al., 2009; Loganathan et al. 2009)

Sampling of Illicit Drugs in Surface Waters

The techniques used for collecting samples of surface waters, or of any environmental matrix, for the detection of illicit drugs are no different than would be used for any other chemical class. Illicit drugs, like many OTC and prescription pharmaceuticals, can have vast differences in their chemical structure resulting in a wide range of water solubility, photolytic stability, and other physicochemical parameters. The specific parameters, important in determining the storage and extraction conditions, have little to no impact on the selection of the sample collection method.

The decision on the sampling method to use is constrained by the type of information needed to answer a specific hypothesis and by the available resources (both logistical and financial). Instantaneous or time-integrated, whole water or dissolved (filtered), one sample or replicates, and how much and what types of quality control measures will be used are all options that need to be considered as part of the sample collection plan (Alvarez and Jones-Lepp, *in press*). The development of a sound sampling plan will help eliminate problems in the field and ensure a representative sample will be collected to meet the needs of the study.

Sampling Techniques

The collection of surface water samples generally falls into two classes of methods: active or passive. Active sampling techniques involve physically taking a sample either by manual or automatic means. Grab sampling methods are among the most common of active methods which in the most simplistic form is filling a container with water at a specific location. This is performed by "hand-dipping" a container from the shore or boat or by lowering a container into the water from a structure such as a bridge. If discrete samples are desired to be taken from a specific depth in the water column, a variety of systems such as the Kemmerer, Van Dorn, and double check-valve bailers can be used (Lane et al., 2003). Depth and width integrated samples can be collected using specialized samplers which can be moved either vertically or horizontally across a water body. Composite samples are often taken to achieve a representative sample of a larger body of water or to obtain an average water sample over time. Composite samples are generated by combining smaller volumes of water in a single container either manually or by use of an automated sampler. Automated samplers are often used in remote locations or locations were water flow may be intermittent. They can be programmed to take samples at predetermined intervals or be started by an external sensor such as a flow meter or depth gauge.

The majority of the published studies for illicit drugs use a simple grab sampling technique of collecting a 1 L water sample in a glass bottle (Buchberger and Zaborsky, 2007; Huerta-Fontela et al., 2008b; Loganathan et al., 2009). Other studies used automated sampling devices to take 24-hour composite samples of 1-2 L of untreated

WWTP influent (raw sewage) and treated effluent samples (Castiglioni et al., 2006b; Zuccato et al., 2008). Postigo et al. (2008) also collected 24-hour composite samples of influent and effluent samples, but only needed a final sample size of 5 mL due to the use of an on-line solid phase extraction system coupled to a liquid chromatography electrospray tandem mass spectrometer.(Postigo et al., 2008)

Passive sampling techniques are those that require no manual or mechanical means for the sampling to occur. The samplers are placed in the water for a defined period of time and chemical uptake (sampling) occurs by diffusion or partitioning process. Passive samplers have advantages over active samplers in that they can be deployed for extended periods (months) in remote locations; episodic events such as runoff, spills, etc. are not missed; they allow detection of trace concentrations of chemicals that may not be possible with standard 1-2 L sample sizes; and in the case of time-integrative samplers, they provide time-weighted average concentrations of chemicals which are a fundamental part of ecological risk assessments (Alvarez and Jones-Lepp, *in press*).

Time-integrative and equilibrium samplers make up the bulk of the passive sampling techniques. Among these, the semi-permeable membrane device (SPMD), the polar organic chemical integrative sampler (POCIS), solid phase microextraction (SPME), polymer sheets, polymers on glass (POGs), and the Chemcatcher are the most common (Alvarez et al., 2007; Mills et al., 2007). Jones-Lepp et al. (2004) were the first

to demonstrate the utility of passive sampling devices in illicit drug monitoring studies. Since then, three other publications describe the use of passive samplers to sample for illicit drugs (Alvarez et al., 2007; Mills et al., 2007; Bartlet-Hunt et al., 2009). In all of these cases, the POCIS was used as it has the ability to sample chemicals containing varied functional groups over a range of polarities common with illicit drugs. Although many of the other passive sampling devices would be capable of sampling certain drugs, they are much more limited in the range of chemical classes that could be sampled.

Handling and Storage Considerations

In general, the collection of environmental waters for the detection of illicit drugs should follow common handling and storage protocols. Samples are generally collected in amber glass containers and shipped chilled (<4-6ºC) via overnight carrier to the laboratory. As with most emerging contaminants, the use of additives as sample preservatives is not required. Upon receipt at the laboratory, the samples should be stored chilled and extracted within 7-14 days. As with all laboratory procedures, storage and holding times for any new chemical should be evaluated prior to sample collection to ensure the integrity of the samples.

Quality Control

The types and amount of quality control used during the field component of a study can vary depending on the data requirements of the study. At a minimum, field blanks should be used to identify any contamination either through direct contact or airborne exposure of the sample. Other quality control samples to be considered include equipment blanks if the same sampling equipment is repetitively used, trip blanks (contaminant-free water samples which accompany the field collected samples from the field to the laboratory but are not exposed to the air), and positive control samples (water samples fortified with the target analytes used to measure any loss or degradation of the analytes due to the handling and storage methods).

Analytical Methods for Illicit Drugs

While this chapter is devoted to detection of illicit drugs in water, we will also briefly mention the analytical methods for environmental media other than water. Many analytical challenges are offered to environmental chemists by the variety of environmental matrices, e.g., sediments, water, plants, biosolids/sludges, and soils, in their quest to tease out individual chemicals from these complex matrices. Additives and naturally occurring chemicals can cause substantial interferences during both extraction and detection methodologies. Since most illicit drugs usually occur in the environment at part-per-trillion (ppt) levels, the analytical methods can require intensive separation and cleanup procedures to isolate and concentrate the chemical from the matrix before analysis.

Extraction Techniques

Solid phase extraction (SPE) is the most widely reported method for the extraction of pharmaceuticals and illicit drugs from aqueous matrices. In this section we will look at SPE, as well as large-volume injection (LVI) and direct injection as extraction techniques.(Jones-Lepp, 2006; Loganathan et al., 2009; Chiaia et al., 2008; Banta-Green et al., 2009; Bisceglia et al., 2009)

Solid phase extractions (SPE). The SPE sorbents are chosen for their ability to retain the pharmaceuticals of interest based upon a variety of the physical-chemical properties of the analytes of interest (e.g., pK_a, D_{ow}, polarity). The SPE sorbent most frequently reported for recovery of illicit drugs, is the hydrophobic lipophilic balanced (HLB) sorbent containing cartridges. Mixed cation exchange (MCX) sorbents have also been used. Jones-Lepp (2006) and Loganathan et al. (2009) reported using the HLB [6-mL capacity, 0.2 g, 30-µm, obtained from Waters Corporation (Milford, MA)] sorbent for the extraction of pharmaceuticals and illicit drugs, and recently published the US EPA's pharmaceutical Method 1694 recommends the HLB sorbent cartridges/discs for aqueous extractions of pharmaceuticals.(Jones-Lepp, 2006; Loganathan et al., 2009; USEPA method 1694) However, Boles and Wells (2009), in a review of analytical methods for amphetamine-like compounds, point to a number of analytical studies using both MCX and HLB sorbents.(Boles and Wells, 2009) They conclude, along with van Nuijs (2009), that MCX and HLB are interchangeable as SPE sorbents.(Boles and Wells, 2009; van

296 Nuijs et al., 2009) The choice of one sorbent over another depends on the compounds of
297 interest, and what interferences would be removed.(Boles and Wells, 2009; van Nuijs et
298 al., 2009)
299
300 Large volume injection (LVI). In Chiaia et al. (2008), they report directly coupling a
301 large volume injector (1800 μL) to a tandem mass spectrometer.(Chiaia et al., 2008)
302 Their method allowed them to detect part-per-trillion (ppt) to part-per-billion (ppb) levels
303 of methamphetamine, amphetamine, ephedrine, cocaine, cocaine metabolites (e.g.,
304 benzoylecgonine, norcocaine, norbenzoylecgonine), hydrocodone, oxycodone,
305 methadone, MDMA, MDMA metabolites (e.g., MDA, MDEA, MBDB), LSD, and PCP.
306 Banta-Green et al. (2009) used the LVI technique, directly coupled to a liquid
307 chromatography-mass spectrometry-mass spectrometry (LVI-LC/MS/MS), to determine
308 the utility of community-wide drug testing.(Banta-Green et al., 2009) They surveyed 96
309 WWTPs for the presence of the illicit drugs, and their metabolites, then back calculated
310 the target community's drug use.(Banta-Green et al., 2009)
311
312 Direct injection. Bisceglia et al. (2009) have recently submitted a publication presenting
313 an isotope dilution direct injection (5 μL) method for the simultaneous detection of 23
314 drugs of abuse and their metabolites.(Bisceglia et al., 2009a) They've also submitted a
315 companion publication demonstrating a streamlined hydrolysis procedure for the
316 determination of cocaine and its two major metabolites. Both methods demonstrate low-

level detection limits (e.g., 20 fg for cocaine) with minimal interferences.(Bisceglia et al., 2009a,b)

Pressurized liquid extraction (PLE). Very few papers have been written describing the extraction of illicit drugs from solid matrices. Stein et al. (2008) describe a PLE method for extracting psychoactive compounds from sediments, and Jones-Lepp and Stevens (2007) also describe a PLE method for extracting methamphetamine and MDMA from biosolids.(Stein et al., 2008; Jones-Lepp and Stevens, 2007) Due to the complexity and variable sizes of environmental solids, the samples usually need to be dried, pulverized and homogenized before extraction. Briefly, small amounts of homogenized solid samples (usually < 2 g) are sub-sampled and extracted. Depending upon what matrix and what analytes are being extracted, the proper solvents, pressures and temperatures are chosen.(Stein et al. 2008; Jones-Lepp and Stevens, 2007)

Detection Techniques

Ion Mobility Spectrometry. It is interesting to note that in 1976 Karasek and colleagues used IMS to detect heroin and cocaine at atmospheric pressure.(Karasek et al., 1976) In the 1980's Lawrence further developed IMS to detect other illicit drugs from solid surfaces and for atmospheric sampling.(Lawrence, 1987; Lawrence, 1986). More recently Hill's research group expanded the utilization of IMS to amphetamine, methamphetamine, PCP, morphine, THC, LSD, and heroin, coupling the IMS to a mass

spectrometer for more specificity.(Wu et al., 2000)

Mass Spectrometry (MS). The majority of detection techniques for pharmaceuticals and illicit drugs are liquid chromatography-mass spectrometry (LC-MS) based. To date the only instruments reported in the US for detecting illicit drugs in environmental matrices are mass spectrometers. The reality is 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. There are a variety of mass spectrometers now being used as detectors coupled to liquid chromatographs (LC). Available as mass detectors are ion trap mass spectrometers (ITMS), quadrupole-time-of-flight mass spectrometers (q-TOFMS), triple quadrupole mass spectrometers (QqQ), magnetic sector mass spectrometers, and most recently orbitrap mass spectrometers. A variety of mass spectrometers have been used, and all US researchers have reported using the tandem mass spectrometry (MS/MS) mode when detecting illicit drugs, as well as other emerging contaminants. The MS/MS mode is where a precursor ion [typically a $(M+H)^+$ in the positive mode, or $(M-H)^-$ ion in the negative mode] is formed in the LC/MS source. The ion formed is transported to an area of the MS where it is energized and collided (either in a QqQ, ITMS, q-TOFMS, or a magnetic sector mass spectrometer) subsequently producing product ions. Product ions are typically the loss of various functional groups from the analytes, for example $(M+H-OH)^+$ or $(M+H-CH_3)^+$. Table 1 shows several illicit drugs, their precursor and product ions as reported in the literature.

In the US, Jones-Lepp et al. (2004) used micro-liquid chromatography-electrospray/ion trap mass spectrometry (μ-LC-ES/ITMS) to assess and detect four prescription drugs (azithromycin, fluoxetine, omeprazole, levothyroxine) and two illicit drugs (methamphetamine and MDMA) in wastewater effluent.(Jones-Lepp et al., 2004) Chiaia et al. (2008) and Banta-Green et al. (2009) coupled LVI to a tandem mass spectrometer (triple stage quadrupole) to accurately identify and quantify a variety of illicit and prescription drugs and their metabolites.(Chiaia et al., 2008; Banta-Green et al., 2009) Bartelt-Hunt et al. (2009) and Bisceglia et al. (2009) used a QqQ to accurately identify and quantify a variety of prescription drugs, non-prescription drugs (e.g., DEET, caffeine), and the illicit drugs, methamphetamine, cocaine, MDMA, etc.(Bartelt-Hunt et al., 2009; Bisceglia et al., 2009)

Accurate illicit drug identification. When using LC-MS techniques for identifying known and unknown chemicals, it cannot be emphasized enough that the analyst must use a MS/MS technique in order to accurately identify analytes. For example, MDMA and caffeine while having different molecular weights have overlapping product ions (mass 163 m/z). However, they have different precursor to product pathways. MDMA with a molecular weight of 193 m/z, forms 194 m/z, $(M+H)^+$, forming the predominant product ion, 163.0 m/z, $(M–CH_3NH_2+H)^+$, using collision induced dissociation (CID). While caffeine having a molecular weight of 194 m/z (one amu different from MDMA), forms 195 m/z, $(M+H)^+$, and under CID, forms predominantly the product ion 138 m/z, $(M-CH_3NCO)^+$, with mass 163 m/z also formed, but less abundantly. Therefore, if an

analyst was monitoring the 163 m/z ion channel, and detected 163 m/z, near or at the same retention time as caffeine, they might misidentify that compound as MDMA, when in fact it is caffeine. Another example would be between methamphetamine and n,n'-dimethylphenethylamine (DMPEA, a widely-used industrial chemical, used as a flavoring agent). These two chemicals are isobaric ions of each other, both have exactly the same molecular mass (149.0 m/z), but are slightly different in chemical structure. Fortunately, under CID LC-ESI MS/MS conditions, these two chemicals form unique predominant product ions, 119 m/z $(M+H–CH_3NH_2)^+$, and 105 m/z $(M+H\text{-}N(CH_3)_2)^+$. However, both compounds also form 91 m/z as a secondary product ion $(M+H\text{-}CH\text{-}N\text{-}(CH_3)_2)^+$. If a researcher chose to monitor mass 91 m/z, instead of 119 m/z, for methamphetamine (and there are those who have reported doing so in the literature) then a false positive for methamphetamine could occur. Therefore, it is important that the proper product and transition ions are chosen to ensure specificity and accuracy.

Occurrence of illicit drugs in US waterways

Jones-Lepp et al. (2004) report detecting both methamphetamine and MDMA (Ecstasy) in the low ppt range from two sewage effluents, one in the southwest and the other in the southeast regions of the US.(Jones-Lepp et al., 2004) Jones-Lepp reported finding in 2006 methamphetamine at two sites, one from an urban creek in Las Vegas, Nevada and the other in the State of Maine, US. Methamphetamine was detected at 5 ng/L in the urban creek, which is surrounded by homes that were on septic tanks.

Methamphetamine was also detected at 7 ng/L at the sewage effluent outfall of a large WWTP in Maine.(Jones-Lepp, 2006) Chiaia et al. 2008, reported detecting methamphetamine at five of the seven WWTPs sampled from throughout the US, with concentrations ranging from 10 to 2000 ng/L, and MDMA at five of the seven plants, with concentrations ranging from 3 to 70 ng/L.(Chiaia et al., 2008) Chiaia et al. (2008) also reported finding cocaine at all seven of the WWTPs sampled (ranging from 10 to 860 ng/L), as well as the prescription opiates: hydrocodone, oxycodone, and methadone. Bartelt-Hunt et al.(2009) sampled eight sites across the State of Nebraska (USA) for a variety of pharmaceuticals and methamphetamine.(Chiaia et al., 2008; Bartelt-Hunt et al., 2009). They detected methamphetamine at seven sites, except one upstream from the Lincoln WWTP, ranging from 2 ng/L to 350 ng/L (effluent from Omaha WWTP). The lower levels of methamphetamine were detected not only in WWTP effluents, but also in streams that were upstream from large city WWTPs.(Bartelt-Hunt et al., 2009) This finding can possibly indicate the presence of clandestine drug labs, as well as input from septic tank leakages into these feeder streams. Banta-Green et al. (2009) sampled 96 WWTPs effluents from across the State of Oregon (US) for methamphetamine, MDMA and cocaine.(Banta-Green et al., 2009) At all 96 WWTPs methamphetamine was detected, while MDMA was detected at less than ½ of WWTPs, and benzoylecgonine (a cocaine metabolite) was primarily detected in the urban WWTPs effluents.(Banta-Green et al. 2009) Bisceglia et al. (2009b) reported detecting methamphetamine: average of 200 ng/L; MDMA: average of 20 ng/L; cocaine: average of 800 ng/L; and several metabolites of MDMA and cocaine, from the effluent of the Back River WWTP (a large urban,

Baltimore, Maryland, WWTP serving nearly 1 million people).(Bisceglia et al., 2009b)

A recent, extensive study [conducted by Jones-Lepp (EPA), Alvarez (USGS) and Sanchez (University of Arizona, Yuma AgriculturalCenter)] along the Colorado river shows the input of illicit drugs into the Colorado River from various sources. The Colorado River, USA, is the main water source (e.g., drinking, agricultural, industrial) for millions of people living in the Southwestern part of the United States (e.g., Nevada, Arizona, California, Utah, Colorado) and western Mexico. Samples were taken throughout the Colorado River Basin, from the Upper Basin, starting at Glenwood Springs, Colorado, to the Lower Basin, ending in Somerton, Arizona (see figure 2). Using a modified version of the method (Oasis MCX, instead of Oasis HLB, SPE cartridges) established by Jones-Lepp (2006), methamphetamine, MDMA and pseudoephedrine were detected in most of the effluents of the WWTPs sampled, and at three different non-WWTP sites (Crystal Beach, AZ; New River, CA; Cedar Pocket, AZ), see table 2.

Pseudoephedrine (a similar in structure to methamphetamine and MDMA) was detected in the Virgin river (a tributary of the Colorado River) at Cedar Pocket, AZ. Cedar Pocket is located along the Virgin River, and is approximately 18 km downstream from the St. George, UT, WWTP, which empties into the Virgin River. One possibility for detection at this site is may be the negative log D_{OW} = -1.85, at pH 7, indicating that it is more hydrophilic, and therefore more likely to stay in the water column, as compared

to methamphetamine and MDMA.

Methamphetamine, at 220 ng/L, was detected in the New river, CA. The New river, is interesting, geographically speaking, as the New river flows out of Mexicali, Mexico, and back into Calexico, United States, to the Salton Sea sink in California. There are raw human waste sources, and illegal methamphetamine manufacturing laboratories, along the New river, starting in Mexico, and back along to the Salton Sea, that could contribute this drug into the waterway.(personal communication with anonymous US Border Patrol officer)

The third non-WWTP site, was off-shore, in the middle of the Colorado river, near Crystal Beach, AZ. This site was sampled three times, May, July, and November of 2007, and methamphetamine and MDMA were detected only once, at 22 and 36 ng/L, respectively, in the July 2007 sample.

Conclusions

We can see from this chapter, that there are several viable methods available, depending upon the analytical need, to separate, concentrate, quantify and reliably detect these compounds. The caveat is that mass spectrometry is the only definitive detection method, and it must be used in the MS/MS mode to ensure accurate detection of not only the illicit compounds, but other emerging contaminants. Papers showing the detection of

illicit drugs in the USA are still few in number (see table 3). However, we can discern from these few studies that illicit drugs, and their metabolites, are making their way into US waterways. There are potential ecotoxicological and sociological ramifications from these findings not yet addressed. Lacking are the ecotoxicological studies to determine whether the levels of illicit drugs detected are of significance to both ecological and human health, both for acute and chronic exposures. It is of socioeconomic significance that, using the methods outlined in this Chapter, researchers have been able to demonstrate the utility of back-calculating from the amounts of illicit drugs found in sewerages, and WWTP effluents, to community usages.(Banta-Green et al., 2009)

Concluding, the methods and approaches presented in this Chapter to detect illicit drugs will provide information needed for developing a framework for exposure and ecotoxicological studies to ensure accurate risk assessments for future regulatory efforts.

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669 Table 1. Several common illicit drugs and their precursor and product ions formed by ESI-MS/MS.

Illicit drug molecular weight (CAS #)	Precursor ions	Product ions	LODs	Reference
Methamphetamine 149.3 amu (537-46-2)	150.0 $(M+H)^+$	119 $(M+H-CH_3NH_2)^+$ 91 $(M+H-CH(CH_3)NH(CH_3))^+$	1.5 ng 1.5 ng/L	Jones-Lepp et al. 2004 Chiaia et al. 2008
MDMA 193.1 amu (69610-10-2)	194.1 $(M+H)^+$	163 $(M+H-CH_3NH_2)^+$	1.0 ng 1.0 ng/L	Jones-Lepp et al. 2004 Chiaia et al. 2008
Cocaine 303.4 amu (50-36-2)	304.1 $(M+H)^+$	182.3 $(M+H-C_7H_5O_2)^+$	2.0 ng/L 20 fg	Chiaia et al. 2008 Bisceglia et al. 2009
LSD 323.4 amu (50-37-3)	324.4 $(M+H)^+$	223.3 $(M+H-C_5H_{11}NO)^+$	0.5 ng/L	Chiaia et al. 2008
PCP (1-(1-phenylcyclohexyl)piperidine) 243.4 amu (77-10-1)	244.2 $(M+H)^+$	159.4 $(M+H-C_5H_{11}N)^+$	2.5 ng/L	Chiaia et al. 2008

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676 Table 2. Concentrations of methamphetamine, MDMA, and pseudoephedrine from Colorado River Basin

Sampling site	Sample type	Amount detected ng/L		
		Methamphet.	MDMA	Pseudoephedrine
Grand Lake, CO (headwaters)	CR	ND	ND	ND
Glenwood Springs, CO	WWTP	253	74	ND
Glenwood Springs, CO	CR	ND	ND	ND
Roaring Fork, CO	CR	ND	ND	ND
Grand Junction/Fruita, CO	CR	ND	ND	ND
Moab, UT	WWTP	ND	ND	ND
Moab, UT	CR	ND	ND	ND
St. George, UT	WWTP	ND	ND	350
Cedar Pocket, AZ	VR	ND	ND	230
Lee's Ferry, AZ	CR	ND	ND	ND
Las Vegas Wash[1]	LVW	230	ND	ND
Crystal Beach, AZ[2]	CR	ND - 22	ND - 36	ND
Lake Havasu, AZ[3]	WWTP	103 (ND – 480)	4 (ND – 17)	330 (ND – 780)
Yuma, AZ	WWTP	650	ND	ND
Gila River, AZ	GR	ND	ND	ND
Tucson, AZ[4]	WWTP	245	ND	372
Imperial Diversion Dam, AZ	CR	ND	ND	ND
Somerton, AZ	WWTP	84	ND	ND
New River, CA	NR	221	ND	ND

677 ND = not detected. Sample Type: CR = Colorado River; GR = Gila River; LVW = Las Vegas Wash below convergence of
678 three WWTPs effluents; NR = New River; VR = Virgin River; WWTP = wastewater treatment plant;
679 [1] Average from 2 sampling events; [2] Range of concentrations of 3 sampling events (min – max)
680 [3] Average from three WWTPs (Northwest Regional, Mulberry, and Island)
681 over one year, where n = 7 sampling events (min – max); [4] Average of n = 9 sampling events from 02/08 to 07/08.

682 Table 3. Analytical methods and illicit drugs identified in US waterways

Reference	Illicit drugs identified	Extraction method	Environmental media
Chiaia et al. 2008	methamphetamine, MDMA, cocaine, cocaine metabolites	Large volume injection	wastewater
Bartelt-Hunt et al. 2009	methamphetamine	POCIS	wastewater
Banta-Green et al. 2009	cocaine, cocaine metabolites	Large volume injection	sewerage
Bisceglia et al. 2009b	methamphetamine, MDMA, cocaine, MDMA metabolites, cocaine metabolites	Direct injection	wastewater
Jones-Lepp et al. 2004	methamphetamine, MDMA	POCIS	wastewater
Jones-Lepp et al. 2006	methamphetamine	SPE	source water, wastewater
Jones-Lepp et al. 2007	methamphetamine	PLE	biosolids
Khan and Ongerth 2003	methamphetamine	unknown	wastewater
Loganathan et al. 2009	methamphetamine, MDMA	SPE	wastewater

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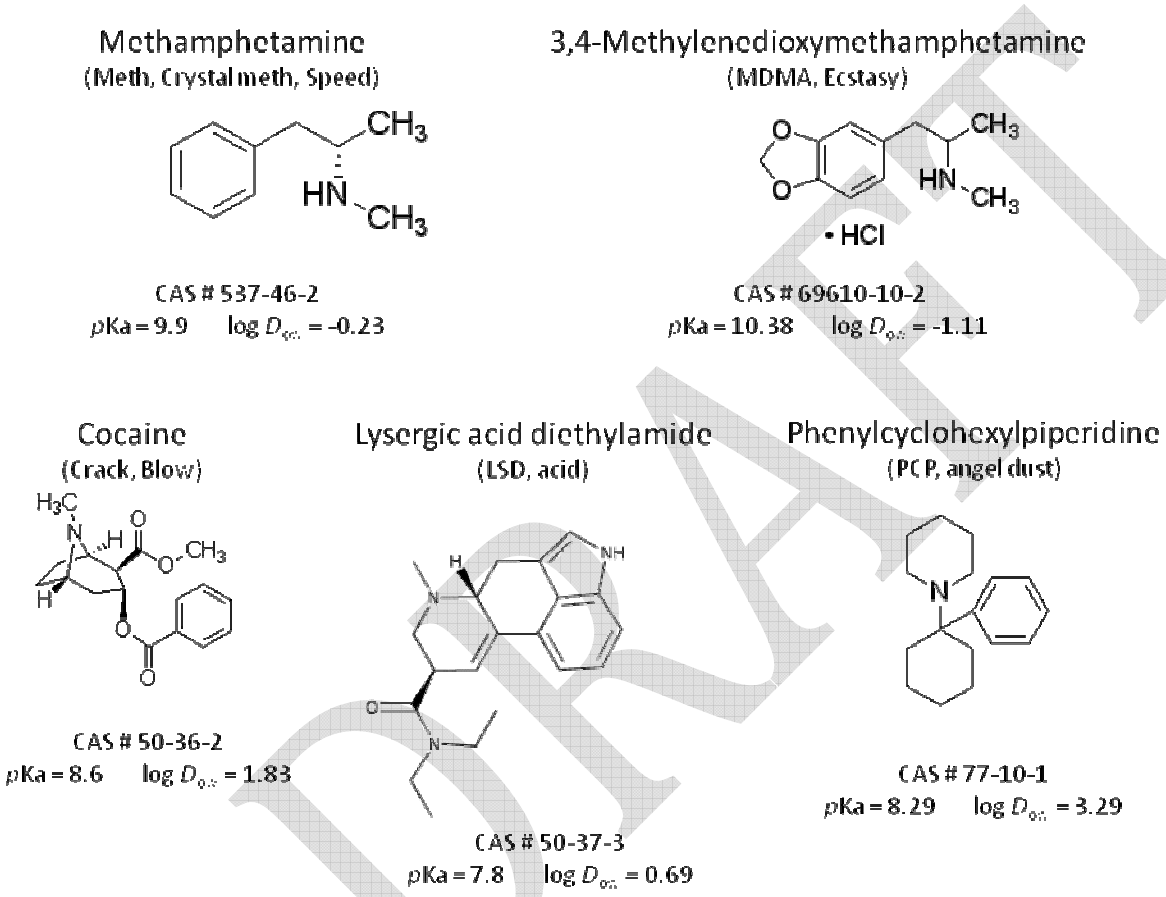
684 Figure 1. Chemical names, common names, structures, and select properties of common illicit drugs.



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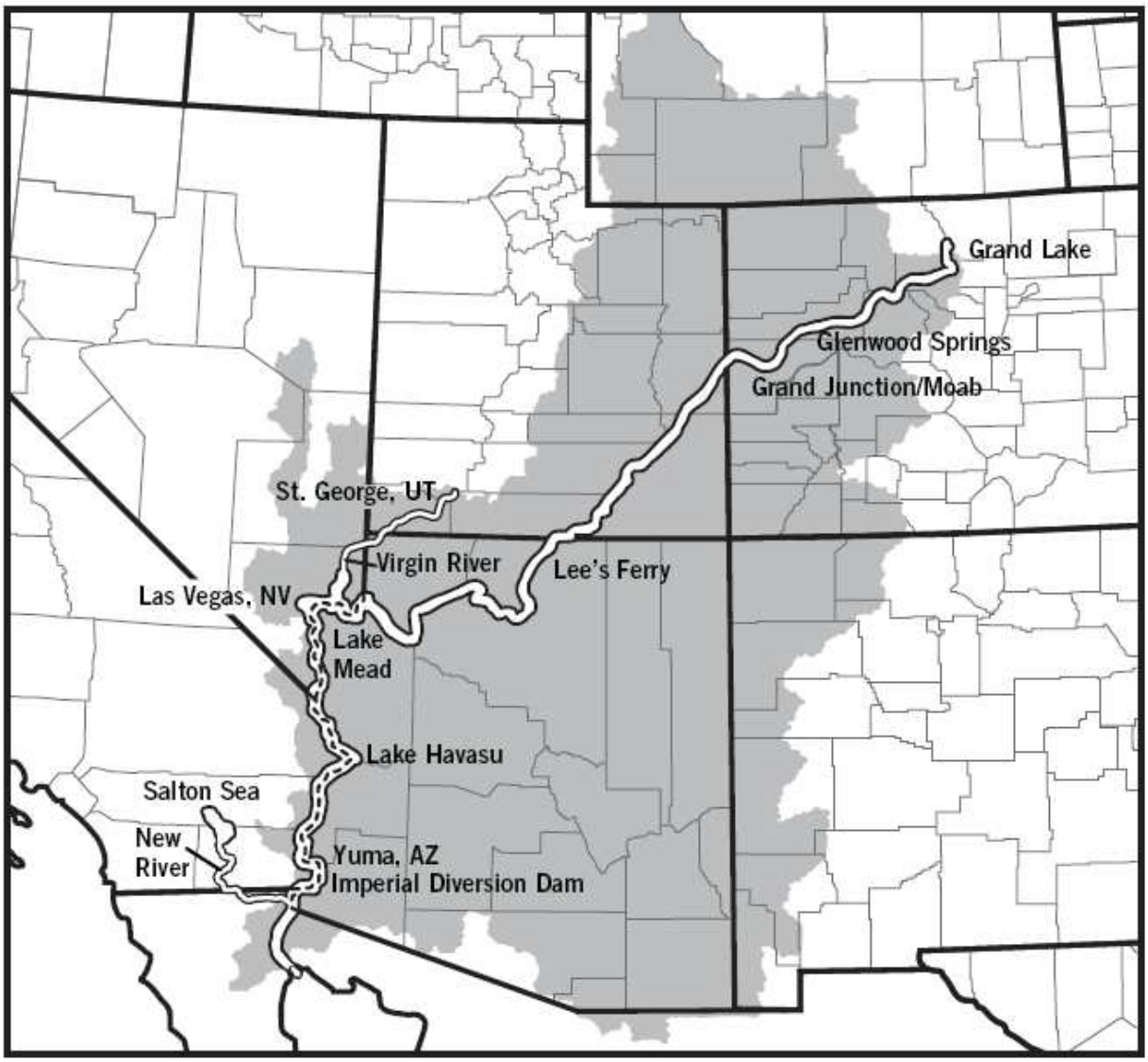
686 *p*ka = acid dissociation constant

687 log D_{ow} = pH-dependent *n*-octanol-water distribution coefficient

688 Figure 2. Colorado river: Upper and Lower Basin.



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