Point sources of emerging contaminants along the Colorado River Basin: Source water for the arid Southwestern United States

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Abstract

Emerging contaminants (ECs) (e.g., pharmaceuticals, illicit drugs, personal care products) have been detected in waters across the United States. The objective of this study was to evaluate point sources of ECs along the Colorado River, from the headwaters in Colorado to the Gulf of California. At selected locations in the Colorado River Basin (sites in Colorado, Utah, Nevada, Arizona, and California), waste stream tributaries and receiving surface waters were sampled using either grab sampling or polar organic chemical integrative samplers (POCIS). The grab samples were extracted using solid-phase cartridge extraction (SPE), and the POCIS sorbents were transferred into empty SPEs and eluted with methanol. All extracts were prepared for, and analyzed by, liquid chromatography-electrospray-ion trap mass spectrometry (LC-ESI-ITMS). Log D_{OW} values were calculated for all ECs in the study and compared to the empirical data collected. POCIS extracts were screened for the presence of estrogenic chemicals using the Yeast Estrogen Screen (YES) assay. Extracts from the 2008 POCIS deployment in the Las Vegas Wash showed the second highest estrogenicity response. In the grab samples, azithromycin (an antibiotic) was detected in all but one urban wastestream, with concentrations ranging from 30 ng/L to 2800 ng/L. Concentration levels of azithromycin, methamphetamine and pseudoephedrine showed temporal variation from the Tucson WWTP. Those ECs that were detected in the main surface water channels (those that are diverted for urban use and irrigation along the Colorado River) were in the region of the limit-of-detection (e.g., 10 ng/L), but most were below detection limits.

Keywords: emerging contaminants, estrogenicity, temporal variation, Colorado River Basin, log D_{OW}

Introduction

Located in the western half of the United States (US) is the Colorado River, which is a major source of water (e.g., drinking, agricultural, industrial) for millions of people living in the southwestern part of the United States (e.g., Arizona, Southern California, Colorado, Nevada, and Utah) and Baja California, Mexico. The focus of this paper was to identify and characterize point sources of a select subset of emerging contaminants (ECs) (e.g., pharmaceuticals, illicit drugs) entering the Colorado River so that this information can be used by water management authorities in their decision making regarding the use, and reuse, of source waters. Samples were collected throughout the Colorado River Basin (CRB), starting in the Upper Basin at Grand Lake, Colorado (the headwaters of the Colorado River), down the Lower Basin, and concluding at the Northern International Boundary (NIB) between California and Mexico (Figure 1).

Nine ECs were chosen for screening, including four antibiotics: three macrolides (azithromycin, clarithromycin, roxithromycin), one lincosamide (clindamycin); one narcotic (hydrocodone); one over-the-counter (OTC) (pseudoephedrine); two illicit drugs (methamphetamine, MDMA or Ecstasy); and one marker of untreated human waste (urobilin). These nine were specifically chosen because of their polar characteristics, amenability to the methodologies used, socially-related reasons, and for their potential for adverse human and aquatic affects. As an example, the four antibiotics were chosen for study because of (1) their widespread use in the US [i.e., azithromycin is the top macrolide antibiotic prescribed in the US, with nearly 49 million prescriptions in 2010 (DrugTopics.com, 2010)], and (2) concern that the presence of antibiotics in wastewater effluents, along with the presence of antibiotic resistant genes (ARg) and antibiotic resistant bacteria (ARb) are creating environmental "hot spots" (Castiglioni et al., 2008; Kemper, 2008; Kim and Aga, 2007; Le-Minh et al., 2010; Loganathan et al., 2009; Merlin et al., 2011; Munir et al., 2011; Rosenblatt-Farrell, 2009; Schwartz et al., 2003; Segura et al., 2009; Seveno et al., 2002). Uncertainty exists as to what will develop from these hot spots, and it has been suggested that

63 more wide-spread and global ARb will arise from these hot spots (Seveno et al., 2002); thereby, rendering
64 modern antibiotics useless or weakened in potency (Felmingham et al., 2007; Knapp et al., 2010). The
65 two illicit drugs (methamphetamine and MDMA) were chosen because of their reported abuse and limited
66 environmental occurrence data in the US (Banta-Green et al., 2009; Bartelt-Hunt et al., 2009; Boles and
67 Wells, 2010; Chiaia et al., 2008; Jones-Lepp et al., 2004; Loganathan et al., 2009). Urobilin, a chemical
68 marker of untreated human waste, was selected because it can be helpful in determining the presence of
69 raw human waste (Jones-Lepp, 2006; Loganathan et al., 2009). The nine emerging contaminants of this
70 study and their chemical formula, CAS #, molecular weight, and log D_{OW} are shown in supplemental table
71 1.

72 The potential for adverse effects from ECs on human health is unknown, but is becoming a
73 concern due to the increasing multi-use and reuse character of wastewater effluent (e.g., snowmaking,
74 golf course irrigation, landscape irrigation, crop irrigation, etc.), and especially where in some cases it is
75 continuously recycled in a closed-loop. This multi-use and recycling of wastewater effluent and the
76 impact upon Southwestern water resources (e.g., Colorado River, Santa Cruz River, Gila River, etc.)
77 increases the potential for cumulative increases of ECs into water supply sources. In the near future,
78 water reuse will become especially important in densely populated arid areas where there is an increasing
79 demand to supply water from limited supplies. Human well-being in a future world will depend more
80 heavily upon this sustainable resource and the characterization of ECs will become important for
81 ecological and human health risk assessments and commodities valuation of water resources (Blasco and
82 Pico, 2009; Young, 2005).

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2. Experimental

2.1 Chemicals

Clarithromycin was obtained from US Pharmacopeia (Rockville, MD, USA). Azithromycin, roxithromycin, and clindamycin were obtained from Sigma-Aldrich (St. Louis, MO) or United States Pharmacopeia (USP, Rockville, MD). Methamphetamine, MDMA, d_5-MDMA, hydrocodone, and pseudoephedrine were obtained from Cerilliant Corporation (Round Rock, TX). Urobilin was obtained from Frontier Scientific (Logan, UT). HPLC-grade methanol was obtained from varying sources [e.g., Burdick and Jackson (Muskegon, MI); EK Industries (Joliet, IL); JT Baker (Phillipsburg, NJ)]. ACS reagent grade acetic acid, glacial and HPLC-grade methyl tert butyl ether (MTBE) were obtained from VWR (West Chester, PA). Acetonitrile was obtained from Burdick and Jackson (Muskegon, MI). Formic acid, ACS reagent grade, was obtained from Anachemia (Rouses Point, NY). Deionized water was produced on-site using a NANOpure^{TM} filtration system (Barnstead, Dubuque, Iowa, USA).

Stock standard solutions were individually prepared from pure standards diluted with HPLC-grade methanol and stored in darkness at $< 4^{o}$ C. A high-level standard mix, used for spiking and calibration standards, was prepared bimonthly in methanol, at concentrations of 10 or 20 ng/µL. Mass spectrometric calibration standards were prepared weekly, from the high-level standard mix, ranging from 0.25 to 2 ng/µL in 99% methanol:1% acetic acid.

2.2 Sampling sites. Sampling sites were chosen from the Upper and Lower Colorado River Basin. Grab water samples, combined with the deployment of passive samplers (polar organic chemical integrative samplers, POCIS), were collected starting at the headwaters of the Colorado River (located on the western slopes of the Rocky Mountains), continuing down the Colorado River, until reaching the NIB at Mexico,

figure 1. Samples also were collected from tributaries (i.e., Green River, Virgin River, Gila River, Santa Cruz River, and the Las Vegas Wash) that reside within the Upper and Lower Basin watershed. While these sites are not along the Colorado River, they do eventually flow into the Colorado River and are part of the CRB watershed. Several of these streams, like the Santa Cruz and Gila River, are mostly ephemeral streams, with their flows resulting from monsoonal storms, winter rains, agricultural run-off, and wastewater treatment plant (WWTP) effluent.

2.3 Sample collection: Grab and Passive sampling techniques

Water samples were collected using either grab sampling or the passive sampling technique, POCIS. Passive samplers were deployed for approximately 30 days at certain collection sites, and collected in conjunction with the grab sampling collection dates for comparison purposes to the grab sampling.

Grab sampling. A pre-cleaned (i.e., acid washed, rinsed with methanol and de-ionized water, and baked at 105^{o} C until dry) 4-L amber glass bottle was submerged under water until filled. The grab samples were placed in a cooler, on ice, transported overnight to the laboratory, and stored at < 4º C until extraction. Extractions usually occurred on the date of receipt of the samples, and were analyzed by liquid chromatograph-mass spectrometry/mass spectrometry (LC-MS/MS), as described in section 2.5.

Passive sampling. Passive sampling devices were used to obtain time-weighted average (TWA) concentration of dissolved organic contaminants at select sites. The POCIS was chosen for this study as

it is designed to sample organic chemicals ranging from hydrophilic to moderately hydrophobic (Alvarez et al., 2004). ECs, such as the pharmaceuticals and illicit drugs targeted in this study, pass through the semi-permeable membrane of the POCIS and are trapped onto a solid-phase sorbent. The sequestered chemicals are then recovered from the sorbent in the laboratory using a simple organic solvent extraction (Alvarez et al., 2008). Briefly, the POCIS were gently cleaned, and the sorbents from each POCIS were transferred into empty SPE cartridges (25 mL capacity) for extraction. Chemical residues were recovered from the POCIS sorbent using 40 mL of methanol. The samplers were deployed for approximately 30 days at select study sites, corresponding to grab sample sites (except for Cibola, where only POCIS was deployed): Lee's Ferry (AZ); Diamond Creek (AZ); Las Vegas Wash (NV); Willow Beach (AZ); Lake Havasu (AZ); Cibola (AZ); Imperial Diversion Dam (AZ); and Northern International Boundary (AZ/CA/Mexico).

The United States Environmental Protection Agency-Las Vegas (USEPA-Las Vegas), using the LC-MS/MS technique described in section 2.5, analyzed the POCIS extracts for ECs. The POCIS extracts were also screened for estrogenic activity using the yeast estrogen assay (YES) screen, described in section 2.7.

2.4 Grab sample preparation and solid-phase extraction

Briefly, water samples were acidified and placed onto an AutoTrace^{TM} solid-phase extraction (SPE) workstation (Dionex Corp, Sunnyvale, CA). The extractions were performed using Oasis MCX SPE (6cc, 150mg) cartridges (Waters Corp., Milford, MA). The eluants were reduced in volume to 0.5 and solvent exchanged with methanol/1% acetic acid, transferred to 2-mL clear glass vials and stored in a refrigerator, at < 4^{o}C until analysis by LC-MS/MS. More details can be found in the Supplementary file.

152 **2.5 LC-MS/MS analysis**

153 A Varian 500MS (Walnut Creek, CA) ion trap mass spectrometer, configured with an electrospray ion
154 source, and a Varian 212-liquid chromatograph, was used for all analyses. Mid-range calibration
155 standards (0.5 and 1 ng/μL) were analyzed at the beginning and end of each analytical day. A volume of
156 5 μL was injected for each standard. Linearity and precision of the daily calibration standards were
157 measured from an initial 3-pt calibration curve prepared and analyzed weekly. A volume of 10 μL was
158 injected for each sample extract. More detailed LC-MS/MS conditions can be found in the supplemental
159 section.

160 Due to potentially interfering materials co-extracted with the ECs, the analyses were performed
161 using the collision induced dissociation (CID) mode for both identification and for calculating the
162 concentration of the analytes of interest. Two to three product ions were used for identification and the
163 most abundant product ion was chosen for quantification. The precursor ion and most abundant product
164 ion that were used to identify and quantify the nine ECs, and their limits-of-detection (LOD, on-column)
165 are listed in supplemental table 2.

166

167 **2.6 Quality Control.**

168 Trip blanks; spike recoveries of each EC in DI water, river water and wastewater matrices, supplemental
169 table 3; and precision and accuracy of calibration standards and sample spikes were determined over the
170 course of the study.

171 For the POCIS, a combination of field blanks and laboratory blanks were used for both the LC-
172 MS/MS analyses and the estrogenic assays. Field blanks were opened to the ambient air during the
173 deployment and retrieval of the passive samplers. Although the chemicals targeted in this study are not

likely to be present in the air, field blanks are important, as other interfering chemicals may have been sampled during these operations.

2.7 Estrogenic assays.

POCIS extracts were screened for estrogenicity using the YES assay (Alvarez et al., 2008), which uses recombinant yeast cells that are transfected with the human estrogen receptor. The recombinant yeast cells also contain expression plasmids carrying a reporter gene (*lac-z*) situated downstream from a promoter sequence, which incorporates an estrogen response element (ERE). Following the binding of a suitable agonist, the yeast cells undergo a cascade of events that result in the release of β-galactosidase into the growth media. The β-galactosidase interacts with a chromogenic substrate (chlorophenol red-β-D-galactopyranoside - CPRG) in the media subsequently producing a color change that can be measured spectrophotometrically. The strength of the color change is a measure of the estrogenic potential of chemicals in the sample. The 96-well test plates were prepared by adding a positive control (17β-estradiol) in the first row and alternating negative controls (200 µL ethanol) and test samples (50 µL extract diluted with 150 µL ethanol in triplicate) in the following rows. All samples and controls are then serially diluted across the test plate. The liquid in each well was allowed to evaporate prior to adding 200 µL of assay medium containing ≈ 4 x 10^7 recombinant yeast cells and CPRG. The plates were gently agitated, sealed, and incubated at 30 ºC for up to 72 hours. Each day, the plates were inspected for the conversion of CPRG in the positive controls to determine the speed of plate development. After 72 hours, the color change was monitored using a plate reader and the absorbance was measured at 540 and 620 nm.

196 **3.0 Results and discussion**

197 **3.1 Occurrence of emerging contaminants in the Colorado River Basin.** Of the antibiotics, only
198 azithromycin, with concentrations ranging from 30 ng/L to 2800 ng/L, was routinely detected in all grab
199 samples of wastewater effluents (with the exception of Moab, UT) that enter into the Colorado River or
200 its tributaries, table 1. The other three antibiotics, i.e., roxithromycin, clarithromycin, and clindamycin,
201 were infrequently detected at lower concentrations in the wastewater effluents. In comparison to other
202 studies done in the US, the azithromycin concentrations in this study are similar to those found by Bartelt-
203 Hunt et al. (2009) in US WWTP effluents from small and large WWTPs in Nebraska (Bartelt-Hunt et al.,
204 2009). Their concentrations detected ranged from non-detect to over 1500 ng/L, in the effluent from a
205 large WWTP in Lincoln, Nebraska, population > 250,000 (Bartelt-Hunt et al., 2009). Murata et al.
206 (2011) reported similar concentrations of azithromycin in Japan , while the levels of clarithromycin
207 detected were slightly higher (Murata et al., 2011). In the Arc river, southern France, Feitosa-Felizzola
208 and Chiron (2009) reported no findings of azithromycin, but very high levels of clarithromycin in
209 comparison to this study (Feitosa-Felizzola and Chiron, 2009). Lin et al. (2008) report concentrations of
210 azithromycin, in Taiwan, consistent with this study, but much higher levels of clarithromycin and
211 clindamycin (Lin et al., 2008). Very low levels of azithromycin, as compared to this study, were detected
212 in several WWTPs located in the Ebro Basin in Spain (Gros et al., 2007). However, it is difficult to
213 compare antibiotic usage across countries, as different countries prescribe different antibiotics, for
214 example roxithromycin is not prescribed in the US, but it is prescribed in Latin America and Europe.

215 Pseudoephedrine and hydrocodone were detected in several wastewater effluents, ranging in
216 concentrations from 120 to 3300 ng/L for pseudoephedrine, and 330 to 900 ng/L for hydrocodone, table
217 1. Hydrocodone was not screened for as an emerging contaminant until half-way through the study time
218 period (2007-2009); therefore, many sites do not have collection data for this compound. Postigo et al.

(2008) found similar levels of ephedrine/pseudoephdrine in waste water effluents in Spain (Postigo et al., 2008).

The illicit drugs, methamphetamine and MDMA (Ecstasy), were detected in several WWTP effluents. The concentrations of methamphetamine ranged from non-detect to 570 ng/L in WWTP effluents, while MDMA concentrations ranged from non-detect to nearly 100 ng/L, table 1. These values are consistent with what others have reported being detected in US effluents (Bartelt-Hunt et al., 2009; Chiaia et al., 2008).

The raw human waste marker, urobilin was detected in several WWTP wastestreams, as well as at the New River site, table 1. The presence of urobilin, along with the presences of human-use drugs/metabolites, can be good indicators of raw human waste (Jones-Lepp, 2006) and identification of these indicators has been used extensively by USEPA's Region 1 to detect and document water quality violations and enforcement actions resulting in the elimination of millions of gallons per year of raw sewage from storm water outfalls (Borci, 2012). Usually, most WWTPs do not discharge urobilin if they are operating properly, and if there are no storm surge overflows. However, high storm sewer overflow can severely impact a WWTP's ability to remove urobilin; and hence, harmful bacteria.

There were five sites that were not wastewater streams where ECs were detected: Cedar Pocket (AZ), Las Vegas Wash (NV), Lake Havasu (AZ), Imperial Diversion Dam (IDD) (AZ), and New River (CA). Pseudoephedrine was detected at 290 ng/L at Cedar Pocket (AZ), this amount was approximately 70% of the amount detected upstream in the St. George WWTP effluent (430 ng/L) that was collected on the same day, table 1. Cedar Pocket (site #10 in figure 1) is located along the Virgin River and is approximately 17 km downstream from the St George WWTP (site #9, figure 1). At a flow rate of 8.78 m^3/sec (real-time data for August 5, 2008 from USGS water gauge station (# 09413700) located below both sites near Littlefield, AZ), it takes a few hours for the water to travel the 17 km. Pseudoephedrine is

242 a small molecule, 165 Da, and has a log D_{OW} < 1, e.g., -1.28 at pH 7, it can be expected that the average
243 levels of use and excretion of pseudoephedrine were fairly consistent over a short period of time.
244 Therefore, it stands to reason that the pseudoephedrine detected at Cedar Pocket is from the effluent from
245 the wastewater treatment plant 17km upstream. Log D_{OW} and its importance to environmental occurrence
246 data will be discussed later in section 3.4.

247 Methamphetamine was detected in the Las Vegas Wash grab samples, table 1. This site is located
248 approximately 8 km downstream from the nearest WWTP effluent stream (Henderson, NV), and 15 km
249 downstream from WWTP #1 and WWTP #2 (Las Vegas, NV). All three WWTPs sit along the Las Vegas
250 Wash wetlands area, which ultimately feeds into the Las Vegas Wash and subsequently, Lake Mead and
251 the Colorado River. Again, methamphetamine, like pseudoephedrine, is a small molecule, 149 Da, and
252 has a log D_{OW} <1, e.g., -0.72 at pH 7.

253 In Lake Havasu (AZ), both methamphetamine and MDMA were detected during the July 2007
254 collection event. Lake Havasu (AZ) is a popular southwest recreational site especially during the summer
255 months. The Lake Havasu collection site was upstream from the effluent wastestreams of the Lake
256 Havasu WWTPs. MDMA was detected twice at very low levels (< LOQ, <LOD, but spectrally
257 confirmed), out of seven sampling events, at the IDD site, which is located downstream from Lake
258 Havasu.

259 Roxithromycin and clarithromycin, 110 and 6 ng/L, respectively, were detected at the New River
260 sample site, which was located just inside the US border at Calexico, California, table 1. Roxithromycin,
261 a macrolide antibiotic, while not prescribed in the US, is a widely prescribed antibiotic in Latin America
262 and Europe. Also detected at the New River sample site were methamphetamine, pseudoephedrine, and
263 urobilin (raw human waste marker). The New River is unique in that it is one of the few rivers that flow
264 northwards into the US from Mexico. The New River starts in Mexico, flows through the city of

Mexicali (Mexico) across the US border, through Calexico (US) and numerous agricultural fields before it empties into the Salton Sea, CA (US). There are municipal wastestreams, raw waste, industrial, and agricultural wastes all entering the New River at various points along the river, both inside Mexico and in the US.

3.2 Passive sampling (POCIS). In 2008 and 2009 field studies the POCIS was deployed at a few select sites: Lee's Ferry (AZ), Diamond Creek (AZ), Las Vegas Wash convergence (NV), Willow Beach (AZ), Lake Havasu (AZ), Cibola (AZ), IDD (AZ); and NIB (AZ/CA/Mexico); to examine and compare the analytes detected between POCIS and grab sampling. POCIS analytes were measured as total ng per POCIS, and then back-calculated using flow rates and uptake rates for correction to ng/L values (Alvarez et al., 2004). ECs were detected at only three sites using the POCIS: Las Vegas Wash (NV); Willow Beach (AZ); and NIB (AZ/CA/Mexico), table 2.

3.3 Comparison of grab and passive sampling. Grab sampling has limitations in that when a sample is taken, it is a "snapshot" of what contaminants are present at that particular moment in time. There is always the vulnerability of collecting a sample just before, or after, contaminants pass by through the water column, and leading to a false negative finding. To test this hypothesis, POCIS were deployed at select sites concurrently with the collection of grab samples. In 2008, at two of the sites, Lee's Ferry and IDD, there were no chemicals detected in either the POCIS or grab samples. At the Las Vegas Wash convergence sample site, several analytes were detected in the POCIS extracts whereas only methamphetamine was present in the grab sample. In 2009, the numbers of chemicals detected in the POCIS and grab samplers were similar. Comparing the number of analyte detections indicate that the POCIS did a better job of identifying the occurrence of these chemicals than the grab samples did.

289 However, the estimated water concentrations were generally lower in the POCIS.

290 Direct comparison of the results between the two sampling techniques should not be made
291 without first understanding the differences in the information provided by both techniques. Grab samples
292 provide a snapshot of the concentration in the water at that exact location and time. Passive techniques
293 provide an integrated view of the concentration of analytes in the water over the entire deployment
294 period. In a flowing body of water, the passive techniques may also provide a slightly better view of the
295 overall chemical concentration in a small area as mixing over time will occur. Often the results will be
296 similar, but they should not be expected to be so. It is well documented that areas directly impacted by
297 WWTPs experience temporal changes in chemical concentrations (often throughout a single day) due to
298 changes in human activities (Gerrity et al., 2011; Managaki et al., 2008; Ort et al., 2005). Also of note is
299 that most of the grab samples were collected over weekend periods where there would be an expected
300 greater influx of human activities in popular vacation areas (such as Las Vegas). It is reasonable to
301 assume that concentrations of certain chemicals would be increased compared to the rest of the work
302 week along with the increase in people visiting an area.

303

304 **3.4 Environmental persistence as linked to log D_{OW}.** The release and persistence of ECs into aquatic
305 ecosystems depend upon their physical-chemical properties and the chemical properties and biological
306 characteristics of the water compartment. These include concentration of dissolved/suspended organic
307 matter, solubility, microbial population, physical (e.g., volatilization from and adsorption to suspended
308 solids and sediment), chemical (hydrolysis, photolysis) and biological removal mechanisms (e.g.,
309 microbial degradation, uptake) in addition to flow and other water characteristics (Baughman and
310 Lassiter, 1978). Two important chemical measurements, pK_a and log D_{OW} (the pH-dependent *n*-octanol-
311 water distribution ratio), can provide strong evidence of whether compounds will be in an ionized state,
312 their hydrophobicity, and can help determine whether they will partition into water, biosolids, sediment

313 and/or biological media (Wells, 2006).

314 Most WWTPs in the US are operated between pH 7 and pH 8. Therefore, this range will be
315 considered in calculating log D_{OW} (Wells, 2006), supplemental table 1. For example, the log D_{OW} of two
316 of the ECs measured in this study, azithromycin (an antibiotic) and methamphetamine (an illicit drug), are
317 -0.06 and 1.59 log D_{OW} at pH 7, respectively, and -0.72 and -0.11 log D_{OW} at pH 8, respectively (values
318 calculated using ACD Labs Phys/Chem History program), supplemental table 1. At a log D_{OW} of -1, the
319 D_{OW} ratio is 0.1/1 equivalent to 1 x 10^{-1}; at a log D_{OW} value of 0, the D_{OW} ratio is 1/1 equivalent to 1 x
320 10^{0}; and at a log D_{OW} value of +1, the D_{OW} ratio is 1/0.1 equivalent to 1 x 10^{1}. Above a log D_{OW} of +1,
321 the likelihood of predominance of the chemical in the aqueous phase decreases logarithmically, whereas
322 below a log D_{OW} of -1, the likelihood of predominance of the chemical in the aqueous phase increases
323 logarithmically. Therefore, compounds having log D_{OW} values in the region between -1 to +1 at a pH of 7
324 – 8 would be anticipated to be found distributed in both the water phase and organic phases during water
325 treatment and transport. Indeed, both azithromycin and methamphetamine have been detected in the
326 water column and in biosolids (Banta-Green et al., 2009; Jones-Lepp et al., 2011; Jones-Lepp and
327 Stevens, 2007; Kim and Aga, 2007; Le-Minh et al., 2010; Loganathan et al., 2009).

328 This interaction between aqueous and solid phases can also be understood by looking at the pK_as,
329 as well as the log D_{OW}s. For example, hydrocodone has a pK_a of 8.52 (calculated using ACD/PhysChem
330 software), indicating that it would be 50% charged and 50% neutral at pH 8.52. Because hydrocodone is
331 a base at pH 8 (below the pK_a) it is even greater than 50% charged, therefore, at pH 8 where it is more
332 than 50% charged, it can be concluded from the log D_{OW} of 1.94, that even the ionized form of
333 hydrocodone is still rather hydrophobic. However, because hydrocodone was detected in water samples,
334 hydrocodone can be considered as an example of a base being a hydrophobic ionogenic organic
335 compound (HIOC) (Wells, 2006). In terms of the transport of hydrocodone through a water treatment

plant (which operate at about pH 7-8 in the U.S.), it could be predicted that hydrocodone will be detected in both the water and the sludge phases. Most of the compounds in this study have log D_{OW} values that are < 1, indicating that they would be detected in the water column after release from WWTPs. Those compounds that have log D_{OW} values that are > 1, like the antibiotics and hydrocodone, were still detected in the water column, consistent with the pK_a data. Empirical data from this study supports the log D_{OW} calculations, in that all of the compounds in this study were detected at some level in the effluents from various WWTPs and non-WWTP sources in the CRB.

Of course, in complex natural water and wastewater samples, partitioning due to hydrophobicity/ lipophilicity is not the only physical-chemical force of attraction operating between molecules. Ion-pair formation and irreversible covalent bonding with organic surfaces in environmental media also occur. However, investigation of pseudo-equilibrium partitioning in these systems is a useful predictor of environmental fate and transport, and log D_{OW} (the pH-dependant hydrophobicity) is more appropriate in these instances than log K_{OW}.

3.5 Temporal data. Presented in Figure 2 is a graph showing the temporal variation of azithromycin, methamphetamine and pseudoephedrine from the Tucson WWTP. There is a significant increase in azithromycin starting in late spring (April 2008) and diminishing concentrations by early summer (June 2008). However, azithromycin never entirely goes away due to its constant use through high prescription rates in the US, (DrugTopics.com, 2010); thereby, labeling it as a pseudopersistent (Daughton, 2002) compound in the effluent wastestream. For methamphetamine, there is an increase in the summer months, but lower concentrations in the winter and spring. There is a notable increase in pseudoephedrine as late spring arrives, and one can assume more allergies and hayfever are present, therefore increasing the use of pseudoephedrine.

359 Other researchers have used data from WWTP effluents to look at temporal, and spatial variations
360 of different classes of drugs. For example Backe et al. (2011) report on temporal trends of androgen
361 loading from a relatively small WWTP in the US Pacific Northwest (Backe et al., 2011). van Nuijs et al.
362 (2009) reported on both spatial and temporal variations of cocaine and its metabolite, benzoylecgonine, in
363 water samples and WWTP effluents from Belgium (van Nuijs et al., 2009). Using principal component
364 analysis (PCA) Terzic et al. (2010) were able to evaluate, over a 8 month period, the temporal variations
365 of several psychoactive substances and their metabolites from a major WWTP in Zagreb (Croatia) (Terzic
366 et al., 2010). Feitosa-Felizzola and Chiron also show temporal changes, between winter and spring, in
367 antibiotic usage along the Arc river, in Southern France (Feitosa-Felizzola and Chiron, 2009).

368

369 **3.6 Estrogenic assays.**

370 Extracts from the deployed POCISs were screened for the presence of estrogenic chemicals using
371 the YES assay. Six of the eight sites were sampled in both 2008 and 2009. At each of these sites, the
372 measured estrogenicity was greater in 2008 than 2009, table 3. Extracts from the 2008 deployment in the
373 Las Vegas Wash showed the second highest estrogenicity, which was not unexpected as this site is
374 heavily influenced by treated wastewater. However, the presence of high levels of toxic chemicals in the
375 2009 POCIS extracts masked estrogenicity measurements from the Las Vegas Wash. In these extracts,
376 there was an observation of yeast cell death at the highest concentrations of the extract during the YES
377 procedure. The observation of yeast cell death was indicative of toxic chemicals present in the extract.
378 The other site that provided the highest level of response for estrogenicity (2.4) was from one of the Lake
379 Havasu sites (114° 19’ 29” W and 34° 26’ 55” N). It is unknown as to why the response was so great
380 from the 2008 extracts from that site, as the POCIS deployment site was in a remote cove along the
381 Colorado River located approximately 3 km (2 mi) downstream from the nearest WWTP effluents.

382 Overall, the estrogenicity measured at most sites was low; however, of those that measured a response,
383 the measured levels may be approaching biologically-relevant concentrations.

384

385 **4.0 Conclusions.**

386 Increasing demands on scarce water resources in the southwestern part of the United States has
387 forced water authorities to look for alternative water sources. One alternative is the use of treated
388 municipal wastewater. This has led to a growing number of water management entities to utilize
389 wastewater effluent to stretch their water consumptive needs. Effluent has been utilized directly from
390 wastewater treatment plants primarily for nonresidential irrigation and to recharge depleted groundwater
391 resources via percolation ponds or injection wells. Some water authorities treat the wastewater using
392 advanced dual-membrane (microfiltration and reverse osmosis) and ultraviolet technologies and then
393 inject the treated used water into ground water aquifers, and pump it out later for further treatment and
394 use as drinking water, or for non-potable water reuse, e.g., use on golf courses, municipal green spaces,
395 etc. This type of reuse has been practiced in several parts of the United States for more than 30 years.
396 For example, the Orange County Water District, Southern California, high quality water reclaimed from
397 treated used water has been injected into ground water since 1976. Other water providers in the
398 Southwest, such as the Phoenix Active Management Area of central Arizona, also recharge their treated
399 wastewater effluent into groundwater reservoirs. Other entities such as the City of Scottsdale, AZ, which
400 is recognized as one of the largest municipal facilities in the world, treats raw wastewater to potable
401 quality for aquifer recharge. The City of Lake Havasu also uses a new state-of-the-art advanced
402 wastewater treatment facility for groundwater recharge. The goal of the City of Lake Havasu is to take
403 the ultra-treated wastewater and inject it into a specially created underground berm, and after further
404 treatment, to eventually use it as source water for the City of Lake Havasu.

405 Knowing that WWTPs can be a significant source of ECs in the Colorado River and its tributaries
406 will hopefully lead water management authorities to a better understanding of ECs in their source waters,
407 that are used for drinking water. For example, some compounds, like azithromycin, can be thought of as
408 pseudopersistent (Daughton, 2002; Daughton and Ternes, 1999) in that they are always present in the
409 wastestreams due to their wide-spread use by humans. Other compounds with higher water solubilities,
410 such as methamphetamine, MDMA and pseudoephedrine, can travel for several kilometers downstream
411 from the WWTPs, or are introduced during recreational activities on the water resource (e.g., lakes,
412 streams, reservoirs). The temporal variations (Figure 2) in the release of different ECs at different times
413 of the year can also lead to an improved understanding of wastewater treatment technologies that perhaps
414 could be tailored more specifically towards certain classes of compounds.

415 The cumulative impact to human health and aquatic ecosystems from the release of multiple ECs
416 (e.g., antibiotics, steroids, hormones, illicit drugs) into the aquatic environment is uncertain. Most levels
417 of ECs detected in the environment are below the toxicity threshold for an acute effect. However, due to
418 the pseudopersistence of many of the ECs it may be possible to elicit an effect from chronic exposure.
419 For example, Brain et al. (2004) showed that certain classes of antibiotics and other pharmaceuticals
420 elicited a phytotoxic response in aquatic macrophytes (Brain et al., 2004). Kümmerer (2010) points out
421 that targeted ecotoxicological studies of ECs are lacking, and that chronic effects often do not have visible
422 results and can remain hidden for a much longer time (Kümmerer, 2010). Chronic exposure, as well as
423 acute exposure, to ECs will likely be of increasing importance in a water commodities-based future where
424 water reuse, and recycling, will play an ever-increasing role, along with the probability of increasing ECs
425 into source water supplies.

426

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438 Tables

439 1. Concentrations of emerging contaminants collected from Colorado River Basin

440 2. Concentrations of analytes detected from POCIS

441 3. Relative estrogenic potential of chemicals sampled by POCIS measured by the yeast estrogen
442 screen

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445 Figures

446 1. Colorado River Basin Watershed

447 2. Temporal trends azithromycin, methamphetamine, and pseudoephedrine from Tucson WWTP

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Supplemental Data for "Point sources of emerging contaminants along the Colorado River Basin: Source water for the arid southwest"; Tammy L Jones-Lepp[a*], Charles Sanchez[b], David A Alvarez[c], Doyle C Wilson[d], Randi-Laurant Taniguchi-Fu[e]

Grab sample preparation and solid-phase extraction. Water samples were poured into 500 mL volumetric flasks, spikes/surrogates were added, and acidified with 12N HCl until < pH 3. Three grams of sodium chloride were added to each sample, the flask was shaken, and placed onto an AutoTrace™ solid-phase extraction (SPE) workstation (Dionex Corp, Sunnyvale, CA). The extractions were performed using Oasis MCX SPE (6cc, 150mg) cartridges (Waters Corp., Milford, MA) with the following procedure: SPE cartridges were conditioned sequentially with: (1) methanol; (2) DI water; and (3) DI water/5% acetic acid; samples (500 mL) were loaded onto the cartridges at 7 mL/min; cartridges were dried for 15 min; the analytes were eluted using 5 mL of MTBE/methanol (90:10), followed by 5 mL methanol/4% NH_4OH, at a flow rate of 1 mL/min. The eluants were qualitatively transferred to TurboVap™ tubes and reduced in volume to 0.5 mL using a TurboVap™ evaporator (Caliper Life Sciences, Hopkington, MA), set to approximately 10 psi N_2, at 25° C. As the extracts evaporated, they were solvent exchanged with methanol/1% acetic acid, then transferred to 2-mL clear glass vials and stored in a refrigerator, at < 4°C until analysis by LC-MS/MS.

LC-MS/MS analysis. A Varian 500MS (Walnut Creek, CA) ion trap mass spectrometer, configured with an electrospray ion source, and a Varian 212-liquid chromatograph, was used for all analyses. Mid-range calibration standards (0.5 and 1 ng/µL) were analyzed at the beginning and end of each analytical day. A volume of 5 µL was injected for each standard. Linearity and precision of the daily calibration standards were measured from an initial 3-pt calibration curve prepared and analyzed weekly. A volume of 10 µL was injected for each sample extract.

The 500MS was run in the positive ionization mode under the following conditions: ES needle, 5 kV; drying gas, 20 psi and 350° C; housing chamber, 50° C; nebulizer gas, 40 psi; spray shield, 600 V; capillary voltages were set dependent upon the optimized response of the product ions of interest, mass range scanned was dependent upon the precursor ion molecular weight and the product ion range, typically 50 to 300 amu for lower molecular weight analytes, and 150 to 800 amu for higher molecular weight analytes.

Due to potentially interfering materials co-extracted with the ECs, the analyses were performed using the collision induced dissociation (CID) mode for both identification and for calculating the concentration of the analytes of interest. Two to three (or more if produced) product ions were used for identification and the most abundant product ion was chosen for quantification. The precursor ion and most abundant product ion that were used to identify and quantify the nine ECs, and their limits-of-detection (LOD, on-column) are shown in supplemental table 1.

Liquid chromatographic separations were performed using an Ascentis Express C18 (Supelco-Aldrich, Bellefonte, PA) 2.7 µm particle size, 3 cm x 2.1 mm column, coupled with a Varian guard column (MetaGuard 2.0 mm Pursuit XRs 3µm C18). The flow rate through the column was 200 µL/min, with the following gradient elution conditions: initial conditions mobile phase A 100%, hold for 2 min; 3 min gradient to 30% A:70% B, hold for 5 min; 3 min gradient to 100% A, hold for 2 min; end run, 5 min equilibration time between analyses. Compositions of the mobile phases were as follows: (A) deionized water/0.5% formic acid, and (B): 82% methanol/18% acetonitrile/0.5% formic acid.

Quality Control. Trip blanks; spike recoveries of each EC in DI water, river water and wastewater matrices; and precision and accuracy of calibration standards and sample spikes were

determined over the course of the study. The recoveries of the ECs were determined by spiking the sample matrices with surrogates and the analytes of interest before extraction and then comparing the amount detected with the amount spiked. Quantitation of the ECs was based on an external standard method (as established by EPA SW-846 Method 8000), and surrogate spikes (one of which is labeled, d5-MDMA) to correct and calculate recoveries. Determination of the ECs are accurate, at least 2 to 3 product ions were used for confirmation, as well as a retention time window of ± 0.5 sec from the standard retention time.

Due to the possibilities of contamination, either during the field grab sampling events, or during the extraction procedures, a blank DI water (i.e., trip blank) was sent out with every field grab sampling event. Therefore, alongside each batch of grab samples (e.g., 4 to 6 samples) received, one trip blank (DI water), one spike of the matrix received (river water or WWTP effluent), and one duplicate of each of the samples were extracted and analyzed.

For the POCIS, a combination of field blanks and laboratory blanks were used for both the LC-MS/MS analyses and the estrogenic assays. Field blanks were opened to the ambient air during the deployment and retrieval of the passive samplers. Although the chemicals targeted in this study are not likely to be present in the air, field blanks are important, as other interfering chemicals may have been sampled during these operations.

LOD/LOQ. The LODs were calculated by analyzing a 4, or 5, point standard calibration curve (including a blank) for each compound in triplicate, the results are shown in table 1. The slope of the line was calculated using linear regression and 3 times the standard deviation (3δ) of the blank area counts was used to established both the LOD and LOQ (MacDougall and Crummett, 1980). Using 3δ from a linear calibration curve, instead of 3δ of the signal-to-noise, can be a more accurate representation of the detection limits, as outlined in MacDougall et al. (1980). The

standard calibration curves used to establish the LOD were linear, most compounds had an r^2 > 0.99, and all had r^2 > 0.9.

Method performance. The SPE method was tested in DI water, wastewater, and river/well water throughout the study. The nine study compounds were spiked into the various matrices and extracted alongside the samples collected during the study. The results of these spiked samples are reported in supplemental table 3. Not surprisingly for most of the analytes the % recoveries were higher in DI water than the wastewater and river/well water. Additionally, the % relative standard deviations (% rsd) were nominally lower, for most of the analytes, in DI water than in wastewater and river/well water. Both of these findings were not unexpected due to interfering substances that can be found in wastewater, e.g., surfactants, polymers, fats, etc., and in river/well water, e.g., dissolved salts and minerals like calcium, magnesium, iron, and copper. These interfering substances can bind to the SPE packing materials, as well as bind to the analytes of interest, thereby interfering with the extraction process. While the extraction recoveries are low for some of the compounds, at the low level spikes (200 ng/L) all have < 30% rsds (except azithromycin, which is 31%) in DI water the only matrix without interferences. Therefore, non-labeled surrogates were used in matrix to correct for interferences in the actual samples that were analyzed from this study. Since it was difficult, and expensive, to obtain labeled analogs for of the emerging contaminants (ECs), non-labeled surrogates (similar in response/structure) were used to compensate and correct for the low recoveries when calculating the final concentrations. Ideally, the best way to calculate actual recoveries and concentrations to overcome the matrix effects is to use labeled standards and isotope dilution. However, at the beginning of this study (2007) the only deuterated analyte available for the compounds of interest in this study was d_5-MDMA. Since the end of this study (2009) two other labeled standards, from the list of compounds of interest in this study, have become available: d_3-azithromycin and d_3-clindamycin.

The extraction methodology has been changed since this study was completed, and deuterated surrogates are now incorporated into all samples.

Supplemental Table 1. Nine emerging contaminants: formula, CAS #, molecular weight, and log D_{OW}

Compound	Formula	CAS #	Molecular weight Da	log D_{OW}[1]		pK_a[1]
				pH 7	pH 8	
Urobilin hydrochloride	$C_{33}H_{43}ClN_4O_6$	28925-89-5	627.17	-2.34	-2.45	4.5 (MA)
Azithromycin	$C_{38}H_{72}N_2O_{12}$	83905-01-5	748.98	-0.06	1.59	13.3 (MA)
Roxithromycin	$C_{41}H_{76}N_2O_{15}$	80214-83-1	837.04	1.75	2.51	13 (MA)
Clarithromycin	$C_{38}H_{69}NO_{13}$	81103-11-9	747.95	1.71	2.47	13.1 (MA)
Clindamycin	$C_{18}H_{33}ClN_2O_5S$	18323-44-9	424.98	0.33	1.2	12.9 (MA)
Methamphetamine	$C_{10}H_{15}N$	537-46-2	149.23	-0.72	-0.11	10.4 (MB)
MDMA(Ecstasy)	$C_{11}H_{15}NO_2$	42542-10-9	193.24	-0.85	-0.21	10.3 (MB)
Pseudoephedrine	$C_{10}H_{15}NO$	90-82-4	165.23	-1.28	-0.37	9.4 (MB)
Hydrocodone	$C_{18}H_{21}NO_3$	125-29-1	299.36	1.05	1.94	8.6 (MB)

[1] log D_{OW} and pK_a values were calculated using ACD Labs Phys/Chem History program. MB = mostly basic, MA = mostly acidic

Supplemental Table 2. LC-MS/MS experimental conditions (positive ESI mode)

Compound	Precursor ion	Product ion	LOD†	LOD Method ng/L	LOQ Method ng/L
Urobilin hydrochloride	591.3 $(M + H - HCl)^+$	343.3 $[M+H- HCl - 2(C_7H_{10}NO)]^+$	0.014	2.7	3.6
Azithromycin	749.5 $(M+H)^+$	591.4 $(M+H-C_8H_{16}O_2N)^+$	0.013	2.5	4.7
Roxithromycin	859.5 $(M+Na)^+$	755.4 $(M+Na-C_4H_9O_3)^+$	0.020	4.0	10
Clarithromycin	748.4 $(M+H)^+$	590.1 $(M+H-C_8H_{16}O_2N)^+$	0.013	2.6	4.1
Clindamycin	425.2 $(M+H)^+$	377.2 $(M+H-SH-CH_3)^+$	0.028	5.6	14
Methamphetamine	150 $(M+H)^+$	119 $(M+H-CH_3NH_2)^+$	0.114	23	46
MDMA(Ecstasy)	194 $(M+H)^+$	163.0 $(M-CH_3NH_2+H)^+$	0.091	18	26
Pseudoephedrine	166 $(M+H)^+$	148.2 $(M+H-H_2O)^+$	0.138	28	68
Hydrocodone	300 $(M+H)^+$	199 $(M+H-C_5H_{11}NO)+$	0.050	10	25

†as determined using MacDougall et al., guidelines (MacDougall and Crummett, 1980), ng on-column.(MacDougall and Crummett, 1980). Triplicate analyses of 3 to 4 different concentration levels plus a blank, using 3x the standard deviation of the blank area counts and the slope of the line generated via linear regression. * Based on 5 µL injections from the linear regression analyses, and 500 µL extracts from 500 mLs of sample.

Supplemental Table 3. Method Performance: Spike recoveries from DI water, wastewater, and river/well water, pH 3.

	Low Level			High Level						
Compound	Spike	DI water (n = 6)		Spike	DI water (n = 6)		Wastewater (n = 7)		River/well water (n = 6)	
	ng/L	%rec	%rsd	ng/L	%rec	%rsd	%rec	%rsd	%rec	%rsd
Urobilin	200	20	24	1000	32	15	44	35	63	45
Azithromycin	200	23	31	1000	17	20	9	49	20	39
Roxithromycin	400	31	20	2000	16	53	2	37	5	67
Clarithromycin	200	8	27	1000	3	10	12	63	12	53
Clindamycin	400	37	15	2000	46	14	42	37	64	29
Methamphetamine	400	71	7	2000	50	21	41	31	39	44
MDMA(Ecstasy)	400	66	4	2000	59	17	34	55	41	49
Pseudoephedrine	400	73	7	2000	47	15	53	32	51	35
Hydrocodone	400	83	4	2000	54	13	40	49	39 (1)	

(1) Hydrocodone was added late in the study, therefore only two river water sampling points were available for spiking recoveries.

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Table 1. Concentrations of emerging contaminants collected from Colorado River Basin

Sampling Sites	Dates sampled	Analytes ng/L								
		Urobilin	Azithro.	Roxithro.	Clarithro.	Clinda.	Meth.	MDMA	pseudoephedrine	hydrocodone
1	07/20/08	-	-	-	-	-	-	-	-	-
2	07/20/08; 08/06/09	**1400; 340**	**170; 910**	-	-	-	**350; 360**	**96; -**	**1800; 3300**	NA; **910**
3	07/19/08	-	-	-	-	-	-	-	-	NA
4	07/20/08, 08/06/09	-	-	-	-	-	-	-	-	NA
5	07/18/08	-	-	-	-	-	-	-	-	NA
6	07/15/08, 08/07/09	-	-	-	-	-	-	-	-	NA, -
7	07/16/08	-	-	-	-	-	-	-	-	NA
8	07/18/08; 08/08/09	-	-	-	-	-	-	-	-	NA; -
9	08/05/08	-	**150**	-	-	**950**	-	-	**430**	NA
10	08/05/08; 07/19/09	-	-	-	-	-	-	-	**290;** -	NA
11	07/14/08	-	-	-	-	-	-	-	-	NA
12	07/14/08, 08/08/09	-	-	-	-	-	-	-	-	NA; -
13[2]	06/30/08; 07/19/09	-	-	-	-	-	**210; 250**	-	-	NA
14	01/19/11 [1]; 06/19/11 [1]	-	**66; 44**	-	**75;** -	**180;** -	**310; 230**	-	**340; 270**	-
15	11/18/08 [1]; 05/22/11 [1]	- ; **60**	**31; 2800**	-	**40; 130**	**1150; 120**	**370; 83**	-	**3100;** -	NA; **330**
16	06/20/08	-	-	-	-	-	-	-	-	NA
17	06/20/08	-	-	-	-	-	-	-	-	NA
18	02/20/08	-	-	-	-	-	-	-	-	NA
19	02/18/08 [1]	-	**140**	-	-	-	**110**	-	**180**	NA
19	03/19/08	-	**920**	-	-	-	**270**	-	**340**	NA
19	04/10/08	-	**240**	**180**	-	-	**260**	-	**340**	NA
19	04/18/08 [1]	-	**1300**	-	**370**	-	**230**	-	**120**	NA
19	05/26/08 [1]	-	**460**	-	-	-	**470**	-	**400**	NA
19	06/07/08	-	**160**	-	-	-	**570**	-	**1000**	NA
20	05/14/07	-	-	-	-	-	-	-	NA	NA
20	07/09/07	-	-	-	-	-	**< LOD[3]**	**35**	NA	NA
20	11/05/07 [1]	-	-	-	-	-	-	-	NA	NA
21	05/14/07	-	**6**	-	-	-	-	**34**	NA	NA
21	11/07/07	-	**50**	-	-	-	-	-	NA	NA
21	02/25/08	-	**11**	-	**78**	-	**190**	-	-	NA
22	05/14/07 [1]	-	**17**	-	-	-	-	**68**	-	NA
22	07/09/07	**5**	**52**	-	-	**26**	-	**<LOD[3]**	-	NA
22	11/07/07	-	**40**	-	-	-	-	-	-	NA
22	02/25/08	-	**96**	-	-	**550**	-	-	**280**	NA

23	02/25/08	-	-	-	-	-	-	-	-	NA
25	06/06/08 [(1)]; 07/21/09 [(1)]	-	**350; 770**	-	-	- ;**740**	-; **570**	-	-	NA; -
26	10/15/07 [(1)]; 01/21/08 [(1)]	-	-	-	-	-	-	**<LOQ[(3)]; <LOD [(3)]**	-	NA
26	02/20/08-07/21/09	-	-	-	-	-	-	-	-	NA
27	02/20/08; 06/11/08	**31**	-	-	-	-	- ; **83**	-	-	NA
28	06/08/08	-	-	-	-	-	**110**	-	-	NA
29	02/19/08 [(1)]	**32**	-	**110**	**6**	-	**200**	-	**140**	NA

"-" = not detected. NA= Compound was not analyzed for. Azithro. = azithromycin; Roxithro. = roxithromycin; Clarithro.= clarithromycin; Meth. = methamphetamine. [1]Average from duplicates; [2] This collection site is approximately 15 km downstream from the WWTP #1 and WWTP #2. (3) Analyte detected spectral confirmation, but below LOD or LOQ.

Legend for Table 2.

1 Grand Lake, CO	16 Diamond Creek, AZ (CR)
2 Glenwood Springs, CO (WWTP)	17 Willow Beach, AZ (CR)
3 Glenwood Springs, CO (CR)	18 Gila River, AZ
4 Roaring Fork, CO (CR)	19 Tucson, AZ (WWTP) (Santa Cruz River)
5 Grand Junction/Fruita, CO (CR)	20 Lake Havasu, AZ (CR) (upstream of Lake Havasu City development)
6 Green River, UT	21 Lake Havasu, AZ (WWTP #1) (CR)
7 Moab, UT (WWTP) (CR)	22 Lake Havasu, AZ (WWTP #2) (CR)
8 Moab, UT (CR)	23 Lake Havasu, AZ (CR at Lake Havasu City)
9 St. George, UT (WWTP) (VR)	24 Cibola, AZ (CR)
10 Cedar Pocket, AZ (VR)	25 Yuma, AZ (WWTP) (CR)
11 Cammeron, AZ (Little CR)	26 Imperial Diversion Dam (IDD), AZ (CR)
12 Lee's Ferry, AZ (CR)	27 Northern International Boundary (NIB), AZ (CR)
13 Las Vegas, NV (LVW)	28 Somerton, AZ (WWTP) (CR)
14 Las Vegas, NV (WWTP #1) (LVW)	29 New River, CA
15 Las Vegas, NV (WWTP #2) (LVW)	

Sample Type: CR = Colorado River; LVW = Las Vegas Wash; VR = Virgin River; WWTP = wastewater treatment plant;

Table 2. Concentrations of analytes detected from POCIS (only samples with at least one detection are shown)

		Analytes ng/L					
Site Names	Date Deployed	Azithromycin	Methamphetamine	MDMA	Clindamycin	Pseudoephedrine	Hydrocodone
2008							
Las Vegas wash	09/08/08	-	**14**	**0.8**	**9.5**	**12**	NA
2008							
Willow Beach	12/02/08	-	-	-	-	-	**22**
2009							
Las Vegas Wash	06/21/09	**0.5**	**6.5**	-	**26**	-	**71**
Northern International Boundary	07/09/09	-	**2.4**	-	-	-	-

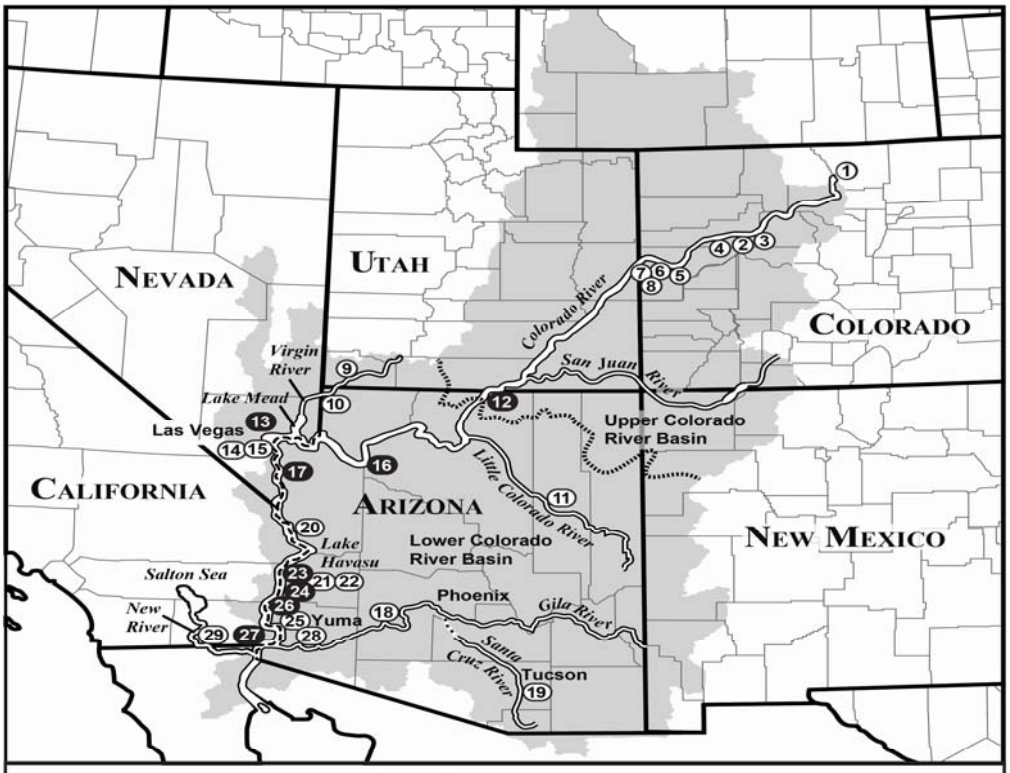
"-" = not detected. NA= Compound was not analyzed for.

Table 3. Relative estrogenic potential of chemicals sampled by the polar organic chemical integrative samplers (POCIS) measured by the yeast estrogen screen (YES).

	2008 (June-September) ng E2/L[a]	2008 (December) ng E2/L	2009 (June-July) ng E2/L
Lake Havasu	2.4	not sampled [b]	trace [c]
Diamond Creek	not sampled	0.73	- [d]
Willow Beach	0.24	0.66	-
Las Vegas Wash	1.9	not sampled	*0.26* [e]
Lee's Ferry	0.04	not sampled	-
Northern International Boundary	not sampled	not sampled	1.2
Imperial Diversion Dam	0.43	not sampled	0.04
Cibola	not sampled	not sampled	0.05

[a] Estimated estradiol equivalents reported in units of nanograms of 17β-estradiol per liter backcalculated from ng E2/POCIS data.
[b] not sampled – POCIS were not deployed during this time period.
[c] estrogenicity was observed above the 99% confidence interval of the blanks, but was below a measurable level.
[d] "-" = not detected
[e] estrogenicity was masked by toxicity in the extract

Figure 1.



Grab Sampling sites

1	Grand Lake, CO
2	Glenwood Springs, CO (WWTP)
3	Glenwood Springs, CO (CR)
4	Roaring Fork, CO (CR)
5	Grand Junction/Fruita, CO (CR)
6	Green River, UT
7	Moab, UT (WWTP) (CR)
8	Moab, UT (CR)
9	St. George, UT (WWTP) (VR)
10	Cedar Pocket, AZ (VR)
11	Cammeron, AZ (Little CR)
12	Lee's Ferry, AZ (CR)
13	Las Vegas, NV (LVW)[2]
14	Las Vegas, NV (WWTP #1) (LVW)
15	Las Vegas, NV (WWTP #2) (LVW)
16	Diamond Creek, AZ (CR)
17	Willow Beach, AZ (CR)
18	Gila River, AZ
19	Tucson, AZ (WWTP) (Santa Cruz River)
20	Lake Havasu, AZ (CR) (upstream of Lake Havasu City development)
21	Lake Havasu, AZ (WWTP #1) (CR)
22	Lake Havasu, AZ (WWTP #2) (CR)
23	Lake Havasu, AZ (CR at Lake Havasu City)
24	Cibola, AZ (CR)
25	Yuma, AZ (WWTP) (CR)
26	Imperial Diversion Dam (IDD), AZ (CR)
27	Northern International Boundary (NIB), AZ (CR)
28	Somerton, AZ (WWTP) (CR)
29	New River, CA

POCIS Sampling sites

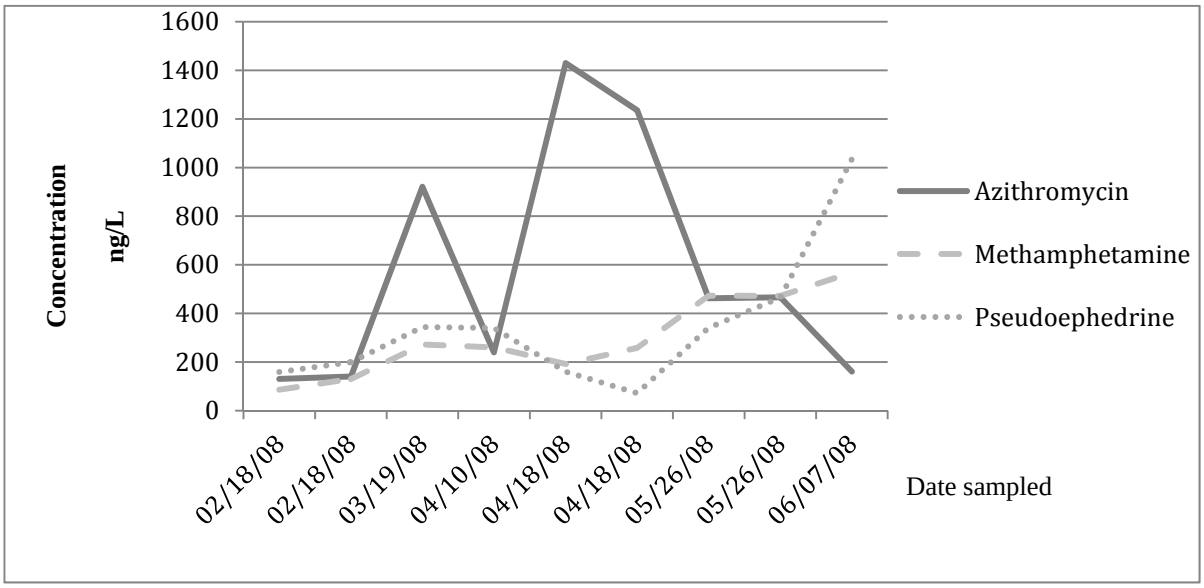
2008

Lee's Ferry
Las Vegas Wash
Willow Beach
Lake Havasu
Imperial Diversion Dam

2009

Lee's Ferry
Diamond Creek
Las Vegas Wash
Willow Beach
Lake Havasu
Cibola
Imperial Diversion Dam
Northern International Boundary

Figure 2.


Concentration
ng/L
1600
1400
1200
1000
800
600
400
200
0
02/18/08
02/18/08
03/19/08
04/10/08
04/18/08
04/18/08
05/26/08
05/26/08
06/07/08
Date sampled
Azithromycin
Methamphetamine
Pseudoephedrine