Fate of Emerging Contaminants in an Effluent Dependent Stream: the Role of Suspended Solids and Sediments (2012AZ492B)

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a. PROBLEM AND RESEARCH OBJECTIVES

Many substances used in domestic households are persistent and pass through conventional wastewater treatment. Among these, chemicals of emerging concern (CECs), including endocrine disrupting compounds (EDCs), are of particular interest. In a 2002 nationwide survey, the USGS measured some of the highest in-stream concentrations of EDCs in the effluent-dependent lower Santa Cruz River (SCR) near Tucson. Targeted testing by the City of Tucson during 2009 and 2010 under their Microconstituent Sentinel Program detected the compounds perfluorooctane sulfonate (PFOS), carbamazepine, and sulfamethoxazole in three groundwater production wells located along the lower SCR (15-20 mi downstream from wastewater effluent outfalls), suggesting that extracted ground water may include a component of effluent origin. Clearly, concern is warranted regarding the presence and fate of CECs in the Lower SCR watershed.

To better understand CEC loadings to the effluent-dependent lower SCR in Tucson, a 2011 investigation by PIs Quanrud and Snyder investigated the presence and fate of a suite of 13 representative CECs during river transport along a 22-mile reach of the lower SCR. A series of groundwater monitor wells located along that same reach was also sampled to assess CECs fate following riverbed infiltration/percolation of effluent. While that study provided substantial new information on transport and fate of selected emerging organic contaminants in the Lower SCR Watershed, it was limited to examining only liquid-phase CECs concentrations and did not assess toxicity or endocrine disruption activity. Many CECs have moderate to high hydrophobicity (high log Kow values) and tend to partition to the solid-phase. Suspended solids in effluent discharged to the SCR are thus a potentially significant additional source of hydrophobic CECs to the Santa Cruz watershed that were not accounted for in previous investigations. CECs may accumulate in riverbed sediments due to deposition of suspended solids as well as by sorption during effluent infiltration/percolation in the riverbed.

The ecological impact of current CECs loading to sediment in the SCR is unknown but it is reasonable to postulate that benthic organisms uptake CECs and that at least some compounds are biomagnified up the food chain. With the expectation of improved river water quality after completion of SCR wastewater treatment plant upgrades in 2015, reestablishment of fish populations, as has already occurred downstream of the newly upgraded Nogales International Wastewater Treatment Plant located on the Upper SCR, may in fact facilitate a greater biomagnification of some CECs to newly re-established aquatic organism populations and higher-level predators (e.g. fish-eating birds and/or mammals).

Here, we assessed endocrine disruption activities in liquid-phase wastewater effluent, suspended solids, and riverbed sediments as a function of downstream travel distance. A combination of bioassays was used to assess estrogenic and androgenic activities: the Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS) reporter gene assays. The present study was motivated by the need to assess the transport and fate of CEC toxicity contribution provided by the solid-phase in an effluent dependent stream, along with the need to establish baseline data in

the Santa Cruz River prior to the 2015 completion of upgraded treatment processes at the two Pima County municipal wastewater treatment facilities that will substantially improve effluent quality and river health.

b. METHODOLOGY

General. A three-pronged sampling approach was performed that included collection of liquid phase, suspended solids, and riverbed sediments at six locations along a 37-km reach of the Lower SCR (Figure 1). Liquid samples (3L) were collected using pre-cleaned and muffled amber glass bottles and filtered within 24 hours of collection using 0.7 μm glass fiber filter membranes (Whatman). Filter membranes were extracted as described below to recover CECs associated with the suspended solids fraction of the samples.

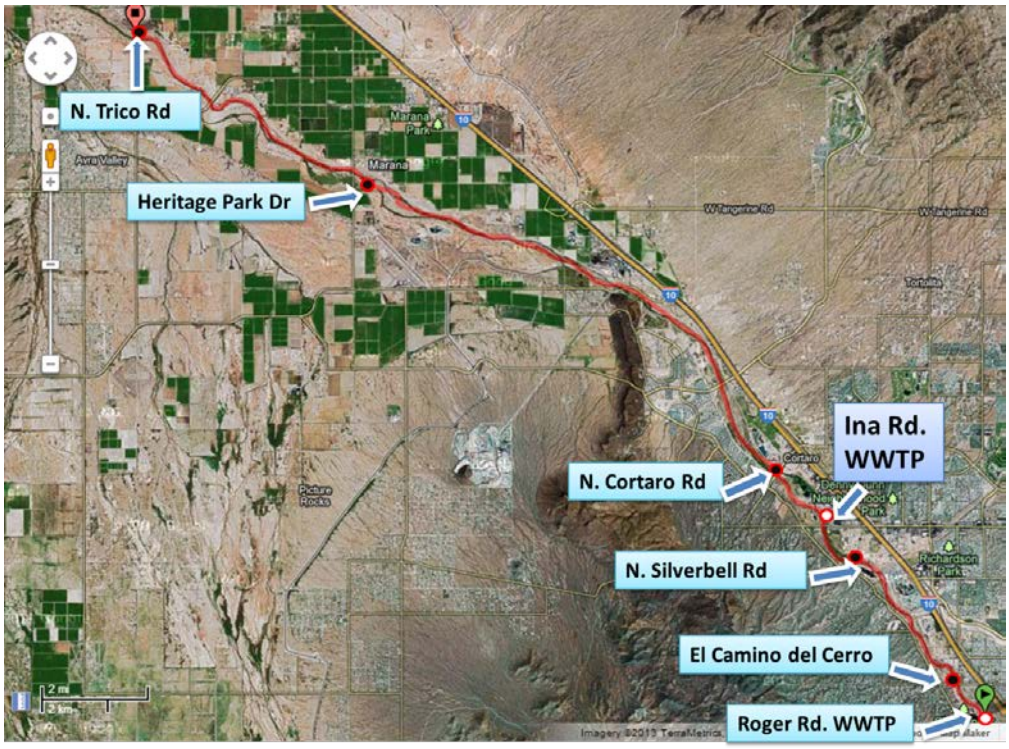

Figure 1. Aerial map showing the six sampling locations along the 37-km reach of the lower Santa Cruz River extending northwest from the City of Tucson, Arizona.

Riverbed sediments were collected proximate to the Roger Road effluent outfall and at five additional locations downstream to Trico Road (Figure 1, Table 1). At each location, riverbed sediments were collected at two depths: 0-3 cm and 10-12 cm using pre-cleaned and muffled amber glass jars. Each sediment sample was a composite composed of at least 2 replicates obtained along a cross section of the river at each location. Sediment sampling was performed before (6-22-13) and after (7-18-13 and 10-13-13) the summer monsoon storm season in order to assess impacts of scouring/deposition on sediment-bound estrogenic activity. Since flow rates in the Santa Cruz can increase substantially during summer stormwater runoff events, which are known to scour and transport riverbed sediments, sediment sampling was performed before and

after the summer rainstorm season to assess associated impacts on sediment-bound endocrine disruption activities.

Table 1. Sampling locations for liquid phase, suspended solids, and riverbed sediments along the 37-km reach of the lower Santa Cruz River near Tucson, Arizona.

Sampling Site Name	Distance downstream, km (mi)	Location
Roger Rd outfall	0.00 (0.00)	32º17'4"N, 111º1'46"W
El Camino del Cerro Rd	1.49 (0.93)	32º17'42"N, 111º2'18"W
N Silverbell Rd.	7.18 (4.49)	32º19'41"N, 111º4'26"W
N. Cortaro Rd.	10.91 (6.82)	32º21'8"N, 111º5'46"W
Heritage Park Dr.	26.75 (16.72)	32º25'31"N, 111º12'57"W
N. Trico Rd.	37.25 (23.28)	32º28'17"N, 111º18'14"W

Sample Preparation/Extraction. All analytical work was performed in laboratories located in the Department of Chemical and Environmental Engineering on the University of Arizona campus. Aqueous-phase samples sometimes require a degree of "cleaning" and analyte concentration, which can be carried out by solid phase extraction (SPE) and elution from the SPE resin in a stepwise methanol gradient. Compounds more hydrophobic than *p*-nonylphenol (log K_{OW} ~ 4.5) tend to be retained on reverse phase resins, even through alcohol elution steps, and can be separated from the estrogens and estrogen mimics in this way. The technique is equally useful for androgen separations. Concentration factors >103 are conveniently obtained by processing initially large water volumes—on the order of a few liters. The *in vitro* endocrine disruption activity tests require an aqueous-phase sample, so that the methanol/water eluent must be evaporated before analytes are redissolved in water. Solid-phase samples like dried sludge or sediment/soil provide a more formidable challenge. Analytes were separated from bulk solids in an adaptation of microwave accelerated extraction (MAE). The MAE procedure developed here is relatively gentle, involving low heats/pressures during 30-min extractions in methanol. Extracts were diluted in ultrapure water, and the methanol water mixtures then processed using normal SPE procedures (above).

Endocrine Activity Assays. In both the yeast estrogen screen (YES) and yeast androgen screen (YAS) procedures, a genetically modified strain of *Saccharomyces cerevisiae* is used to detect and signal the presence of estrogen/androgen agonists and antagonists in environmental samples, wastewater, sludge, etc. A degree of sample preparation is required. The YES (Routledge and Sumpter, 1996) is a reporter-gene assay in which β-galactosidase is produced by the genetically modified yeast strain in the obligate presence of estrogenic compounds. The human *hER-α* gene was used to transform the yeast genome, where it is expressed constitutively. After an estrogen agonist or antagonist enters the yeast cell, it combines with the *hER*-α estrogen receptor protein, forming a complex that binds to the plasmid-borne estrogen receptor element (ERE) leading to transcription/translation of the reporter gene, here *β-gal*. β-galactosidase so produce is capable of cleaving chlorophenol red-β-galactopyranoside (CPRG) into chlorophenol red and galactose. The concentration of the red dye so produced is determined colorimetrically at λ = 570nm after a specified incubation period in the presence of CPRG and compared to a set of standards to determine whole-sample estrogenic activity. YAS procedures are entirely parallel. Differences

between the tests arise from the nature of the genetic modifications to the test organism only. Anti-estrogen and anti-androgen activities can be determined via modest modification of the original procedures (Sohoni and Sumpter, 1998).

Structural differences between the cell envelopes of human and yeast cells and differences in cofactors used for gene expression, have motivated skepticism regarding the applicability of the YES/YAS procedures for determining exogenous stimulation or repression of endocrine regulated activities in fish or humans. Reservations have largely been set aside, however, by direct comparison of the YES/YAS response to known estrogen/androgen agonists with the responses of alternative, mammalian cell assays. Although the YES/YAS procedures are less sensitive than mammalian cell bioassays, this shortcoming is overcome by concentrating samples prior to measurements and more than compensated for by relative procedural simplicity and cost reduction. All of these tests suffer from a singular shortcoming, however, in that each responds only to compounds that are capable of binding to respective steroidal hormone receptor proteins. Other forms of endocrine system disruption cannot be detected in this way.

CEC Analytical Methods

Sample collection and preparation

All samples were collected in pre-cleaned and muffled amber glass bottles. Trace organics were extracted within 24 hours. Samples were filtered through 0.7 µm PALL glass fiber filters, deuterated internal standards were added and then the samples were extracted using Waters Oasis HLB SPE cartridges. HLB sorbents were conditioned with 5 ml of MeOH, 5 ml of MTBE and 5 ml of water. One-half g of EDTA was dissolved in one liter of each source water sample before it was loaded onto the SPE sorbent at 10 ml min^{-1}. Sorbents were dried with N_2 for 40 min before sorbates were sequentially eluted with 3 ml of MeOH, 3 ml of 5% NH_4OH in MeOH, 3ml of ACN and 3ml of MTBE. The combined eluents were evaporated to about 50 µl and re-dissolved in 1 ml 50% aqueous methanol for LCMS analysis.

Analytical

An Agilent 1290 Infinity LC System coupled to an Agilent 6460 Triple Quadruprole LC/MS system using both positive and negative electrospray ionization was used for analysis of CECs (Table 2). Calibration standards were obtained from Sigma Aldrich, except for perfluorohexadecanoic acid (PFHxDA) which was obtained from Matrix Scientific, meprobamate from Cerilliant, and triclosan from Alfa Aesar. Calibration standard solutions were prepared by first making 500ug/mL stock solutions of each standard from the neat solid in HPLC pesticide grade methanol. Subsequent calibration and fortification solutions were prepared by mixing of all standards in methanol at 10ug/ml, followed by successive dilution to obtain the required concentrations. Labeled internal standards were used whenever available, and were purchased from Cambridge isotope laboratories with the exception of 13C4-PFOA, 13C4-PFOS, 13C2-PFHxA, 13C4-PFBA (Wellington Laboratories), 13C6-diclofenac, primidone d5 (Toronto Research Chemicals), and gemfibrozil-d6 (C/D/N) isotopes. All solvents used were of the highest purity available. Methyl tertiary- butyl ether (MTBE), formic acid and ammonium hydroxide were obtained from Fisher Scientific, while acetonitrile and methanol were obtained from Burdick and Jackson.

Table 2. Listing of the 36 CEC analytes that were assessed in the SCR sediments. (Asterick = known endocrine disrupting compound.)

Atenolol	PFBS
Atrazine	PFDA*
Benzophenone*	PFDoA*
Benzotriazole (BTA)*	PFHxDA
Bisphenol A (BPA)*	PFOA*
Caffeine	PFOS*
Carbamezapine	Prednisone
DEET	Primidone
Dexamethasone	Propylparaben*
Diclofenac	Simazine
Diphenylhydramine	Sucralose
Ditiazem	Sulfamethoxazole
Fluoxetine	TCEP
Gemfibrozil	TCPP*
Ibuprofen	Testosterone*
Meprobamate	Triclocarban (TCC)*
Naproxene	Triclosan*
Norgestrel	Trimethoprim (TMP)

c. PRINCIPAL FINDINGS AND SIGNIFICANCE

Estrogenic activity

The concentration of estrogenic activity in secondary effluent discharged from the Roger Rd WWRF into the Santa Cruz River ranged from 1.1 to 1.6 nM EE2 equivalents/L (300 to 450 ng EE2/L) (Figure 3), well above the levels known to elicit serious physiological disruption to any exposed fishes. Since this effluent contains relatively high levels of ammonia nitrogen (on the order of 20-25 mg NH_3 per L), fish populations at present in the lower SCR are essentially nonexistent. Figure 4 shows a comparison of results from the June 22, 2012 sample set for suspended solid and liquid phase components of estrogenic activity during transport along the 37-km reach of the effluent-dependent lower SCR. About 20% of the total estrogenic activity (corresponding to 0.4 nM EE2 equivalents/L (110 ng EE2 equivalents/L) resided in the suspended solid component of the effluent discharged from the Roger Road reclamation facility (Figure 4). It is anticipated that the loading rate of estrogenic activity from the Roger Rd WWRF point source will decrease substantially following completion in 2015 of an upgraded reclamation facility at this location.

For all three sampling events during 2012, the concentration of estrogenic activity in the SCR decreased dramatically during transport downstream from Roger Rd., with both the liquid phase and suspended solid components decreasing by more than 95% after about 7.2 km travel distance downstream from the Roger Rd. outfall (Figures 3 and 4, respectively). It was not possible to assess removal mechanisms of estrogenic activity during this study but responsible processes could include biodegradation, photolysis, and/or settling/sorption to riverbed sediments.

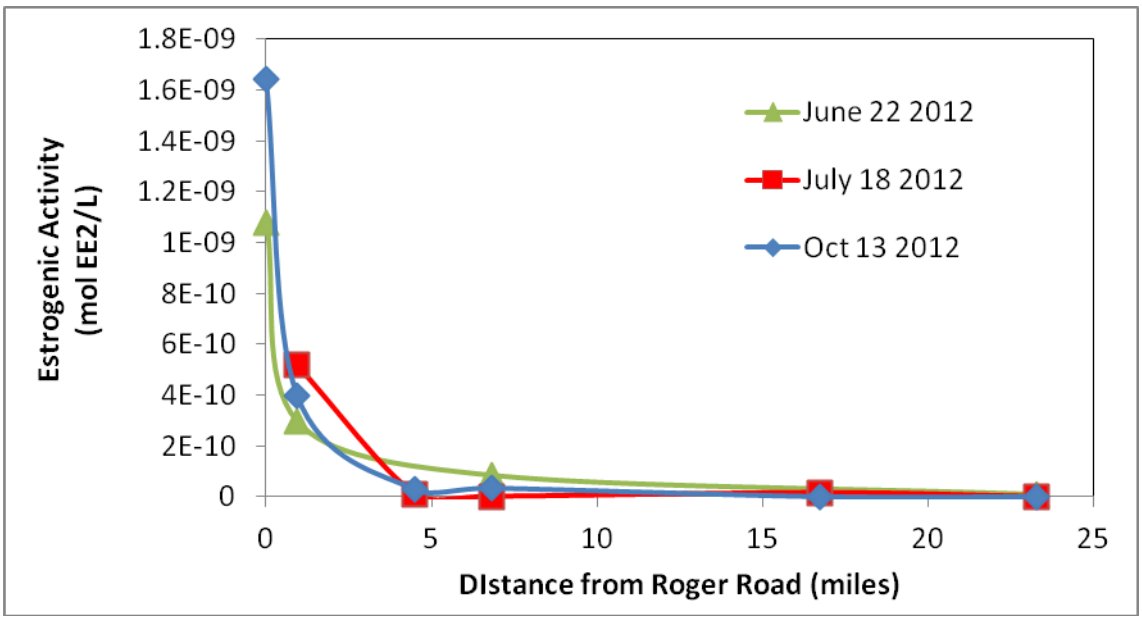

Figure 3. Liquid-phase concentrations of estrogenic activity (moles of EE2 equivalents/L) in water samples collected along the lower Santa Cruz River, Arizona (mile 0 = Roger Rd. outfall).

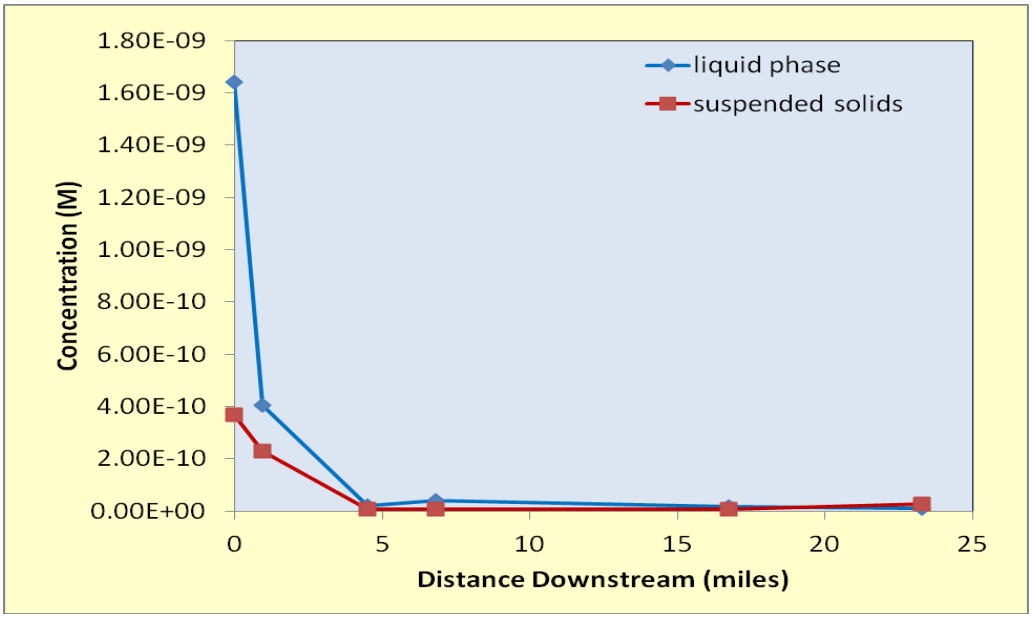

Figure 4. Comparison of estrogenic activity concentrations (moles of EE2 equivalents/L) for the liquid phase and suspended solid sample components along the lower Santa Cruz River, Arizona on June 22, 2013 (mile 0 = Roger Rd. outfall).

Estrogenic activity was detected in some of the 0-5cm depth sediment extracts (Figure 5). The detection limit for estrogenic activity in riverbed sediments was estimated at 2.84 x 10^{-13} M EE2 equivalents/L. Estrogenic activities were highest in the pre-monsoon (June 22, 2013) surface sediment samples collected at the Cortaro Rd. (6.8 mi) and Trico Rd. (23.8 mi) sampling sites.

Estrogenic activity in sediments from these locations was much reduced, or nondetectable, in the two post-monsoon (July 18, October 13) sediment sample sets. These data are consistent with a scenario in which near-surface bed sediments along the study reach are scoured and transported downstream during high flow runoff events in summer, replaced by newly deposited sediments originating from upstream of the Roger Rd. outfall and presumably possessing little or no estrogenic activity (dry riverbed except during storm runoff events). This would thus represent an annual cycle of scour of "contaminated" sediment followed by deposition of relatively cleaner sediment along the effluent-dependent study reach.

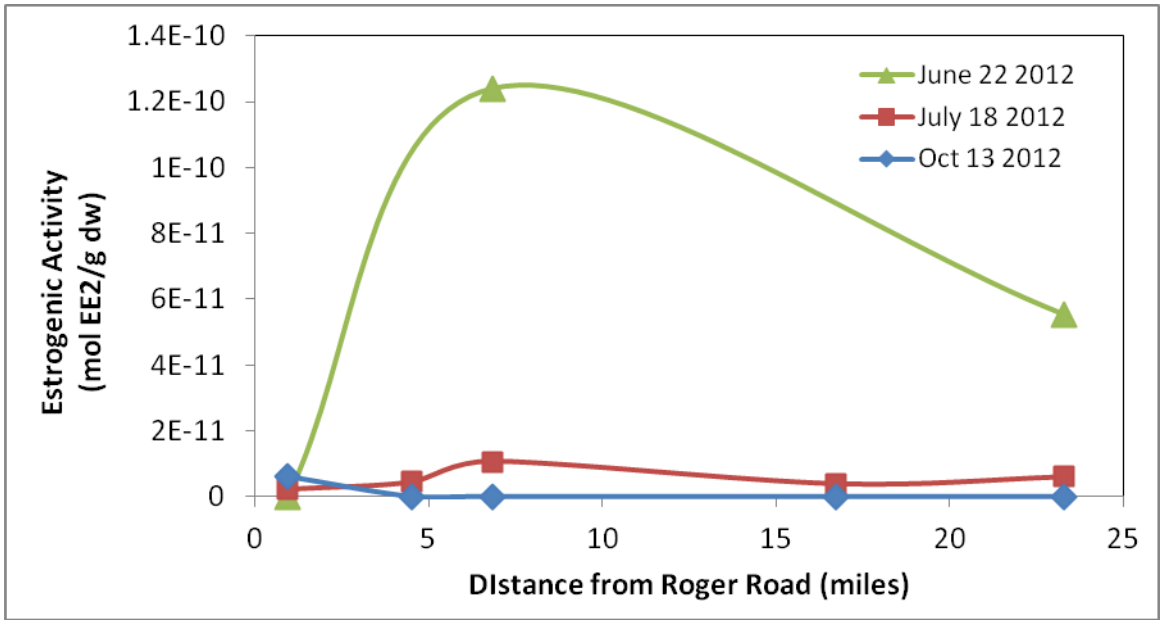

Figure 5. Sediment-bound concentrations of estrogenic activity in the 0-5cm depth sediment samples collected from the lower SCR (mile 0 = Roger Rd. outfall).

Androgenic Activity

Liquid-phase, suspended solid, and sediment extracts were all analyzed for androgenic activity using the YAS bioassay. Suspended solid and sediment extracts all tested negative for androgenic activity. A very small minority of liquid-phase river samples showed very small detections for YAS that could not be reliably quantified.

Chemicals of Emerging Concern

A fourth set of sediment samples was collected along the lower SCR in February 2013 and tested for a suite of thirty six CECs (Table 2). Sixteen of these CECs were detected in the (upstream) sediment sample nearest the Roger Rd outfall; of these, eight CECs (caffeine, TCPP, benzotriazole, triclocarban, trimethoprim, benzophenone, bisphenol A, and triclosan) were detected in sediment extracts obtained from all six riverbed sampling locations (Figure 6) with concentrations ranging from sub parts per billion upwards to almost 100 ppb. Known endocrine disruptors that were detected at the majority of sediment sampling sites included benzophenone, benzotriazole, bisphenol A, TCPP, triclocarban, and triclosan.

The CEC detected at greatest concentration in SCR sediments was caffeine; this result was somewhat unexpected given the modest K_{ow} value for caffeine (log K_{ow} = 0.01). Although there were notable exceptions, sediment-bound CEC concentrations (ng/g) were generally highest towards the upstream sampling sites and decreased as a function of downstream distance (Figure 6).

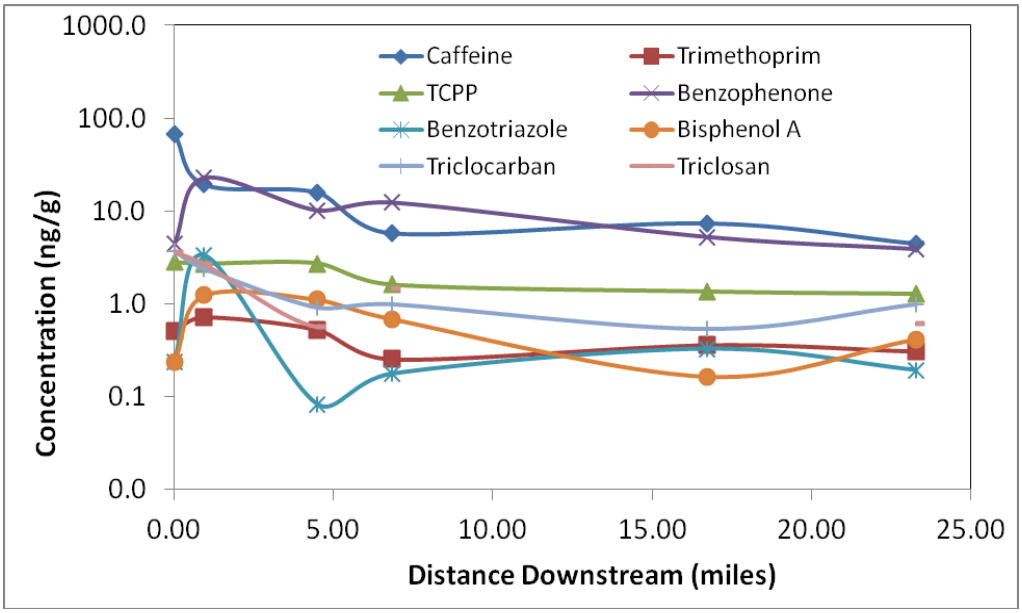

Figure 6. Concentrations (ng/g) of the eight CECs detected at all six SCR sediment sampling locations along the 23-mile (37-km) reach of the lower SCR (mile 0 = Roger Rd. outfall).

Summary of Findings:

Many chemical of emerging concern (CECs) that enter municipal wastewater through domestic use are only partially removed during conventional wastewater treatment. Many of these are innocuous in character (e.g. cholesterol) but they also include endocrine disrupting compounds (EDCs), such as estrone and other estrogenic compounds, at concentrations that are potentially deleterious to continuously exposed aquatic organisms residing downstream from discharge points of municipal effluent. In addition, EDCs and other CECs may accumulate in riverbed sediments via deposition of suspended solids or sorption of liquid-phase CECs during effluent infiltration/percolation in the riverbed. We evaluated the occurrence and fate of EDCs, measured as estrogenic activity, along a 23-mile reach of the Lower Santa Cruz River (SCR) as a function of distance downstream from municipal wastewater reclamation facilities in Tucson. River water, suspended solids, and riverbed sediments were sampled to establish the persistence of toxicity in river/sediments. Sampling was performed before and after the 2012 summer monsoon rainstorm season to assess associated impacts on sediment-bound endocrine disruption activities as consequence of increased river flow rates during summer runoff events. Liquid-phase and suspended solid concentrations of estrogenic activity decreased by more than 95% during in-stream transport along the 23-mile reach of the SCR. Estrogenic activity concentrations in near-surface sediments were found to be highest in the pre-monsoon riverbed samples. Presumably, these sediments were scoured and transported downstream during high runoff events in summer,

replaced by newly deposited (upstream) sediments possessing little or no estrogenic activity. This would thus represent an annual cycle of scour of “contaminated” sediment followed by deposition of relatively cleaner sediment in the riverbed along the effluent-dependent study reach.

References

Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T., Pharmaceuticals, hormones, and other organic wastewater contaminants in us streams, 1999-2000: A national reconnaissance. *Environmental Science & Technology* 2002, *36*, (6), 1202-1211.

Quanrud, D.M., Quast, K., Conroy, O., Karpiscak, M.M., Lansey, K.E., Ela, W., and Arnold, R.G. 2004. Measurement of Estrogenic Activity and Volume Contribution of Treated Wastewater in Water from Wells along the Santa Cruz River, Arizona. *Ground Water Monitoring and Remediation*. 24(2):86-93

Routledge E.J. and J.P. Sumpter. 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environmental Toxicity and Chemistry*. 15(3):241-248.

Sohoni P. and J.P. Sumpter. 1998. Several environmental oestrogens are also anti-androgens. *Journal of Endocrinology*. 158(3):327-339.