Supporting Information for

Combined Sewer Overflows: An Environmental Source of Hormones and Wastewater Micropollutants

By Phillips, P. J., Chalmers, A. T., Gray, J. L, Kolpin, D. W., Foreman, W. T., and Wall, G. R.

Methods

Sampling Network

Samples were collected between November 2007 and December 2008. A schematic diagram of the Main Burlington wastewater treatment plant (WWTP) showing locations of sampling sites is shown in Figure S1. Details of samples collected at the three sites (plant effluent [PE], plant influent [PI], and combined sewer effluent [CSE]) are in Table S1. These details include flow rates, number of 1-L samples composited per sample, and time intervals between sample collection for each composited sample. Twenty two (22) PE samples were collected over 18 different sampling events. Twenty one (21) PI samples were collected over 17 events. Ten (10) CSE samples were collected over 10 events. Samples were generally collected as composites of 1-L grab samples collected over a 24 hour period or during the duration of a storm. All samples were collected as 1-L grab samples, with time between grabs ranging from 1 hour for PE and PI samples to 15 minutes for CSE samples. PE and PI samples were generally collected over 24 hours, although some samples were collected over 8, 12 or 16 hours; Table S1 gives details of each of the samples collected during the study.

Treatment efficiency estimates are based on comparisons of PI and PE samples. There were 17 possible comparisons of PI and PE samples. In most cases (11 of 17), the comparisons were of 24 hour samples collected at both these sites. In two cases, equipment failure limited comparisons to 12 hour samples collected at both sites. In three cases, the 24 hour samples at either the PI or PE site or both sites were divided into sub samples of 8 to 14 hours; these samples were mathematically composited by flow weighting the individual concentrations to determine the concentration over the entire 24 hour period. Finally, in one case a 24 hour sample was collected at the PE site was compared to a 21 hour composite sample collected at the PI site.

Sample Handling

Samples were collected in accordance with standard USGS trace-organic analysis protocols (USGS, variously dated). Most samples were flow-weighted composites that were comprised of 1-L samples that were composited in a stainless steel bucket cleaned according to USGS trace protocols. Hormone samples and some OWC (organic wastewater compound) samples were stored in 1-L HDPE bottles and frozen before analysis. Analysis of reagent-water blanks using the 1-L HPDE bottles by both analytical methods indicated no contamination of analytes when using HPDE bottles. OWC samples were chilled and sent to the laboratory for extraction and analysis just after collection, generally overnight. Samples analyzed for hormone analysis were frozen within 24 hours of collection and stored frozen for 2 to 7 months before extraction and analysis. Limited holding time experiments indicate that the hormone analytes discussed in this report are stable for at least 56 days in spiked reagent water stored frozen, except for cholesterol and coprostanol which exhibited a decrease of no more than 32% relative to day zero concentration, likely from sorptive instead of reactive loss (Foreman and others, 2012). Samples

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from the CSE and PE sites were chemically oxidized during treatment for disinfection purposes (using bromination and chlorination, respectively). Samples collected from these two sites were treated with ascorbic acid as a quenching agent (Valder and others, 2011).

Analytical Methods

Both analytical methods used in this study were developed for whole-water samples. Concentration data for both methods were occasionally reported below the reporting level (RL). Data reporting procedures used by the USGS National Water Quality laboratory (described by Childress and others, 1999) use an approach whereby the laboratory's reporting level (RL) is set to twice the detection level in order to meet a rate of false negatives at no more than 1 percent. Concentration data from both methods reported at concentrations between ½ the RL and the RL are used in this study, but any concentrations reported less than ½ the RL are considered nondetections. Less than 5% of the detections used in this study are between ½ the RL and the RL. Non-detections in figures in this study are plotted at ½ the RL.

Hormone Method

The hormone method is described in Foreman and others (2012). Unfiltered water samples (0.5– 1 L) were extracted by C-18 solid-phase extraction disks (Sigma-Aldrich), and analytes were eluted with 40-mL methanol (Mention of trademarks or commercial products does not imply endorsement by the U.S. Government and is used for informational purposes only). Concentrated extracts were cleaned up using 1-g Florisil[®] SPE columns (Biotage, LLC, Charlotte, NC). Method compounds were derivatized to trimethylsilyl ether or enol-ether derivatives with activated *N*-methyl-*N*-trimethylsilyl trifluoroacetamide (Sigma-Aldrich) and analyzed by gas chromatography with tandem mass spectrometry (GC/MS/MS; Waters Corp.). Compounds were uniquely identified using three precursor-to-product ion transitions. For positive identification, product ion ratios had to fall within specified tolerances established using authentic standards in agreement with the 2002/67/EC European decision (Antignac and others, 2003). Quantitation was done using isotope-dilution procedures with deuterated-labeled isotopes that were either exact or structurally-similar analogs of the method compounds.

Data for twelve of the 20 compounds determined by the hormone method are presented in this article. Of these, the following nine are referred to as the "Hormone" compounds: three estrogens (17-beta-estradiol [E2], estriol [E3], and estrone [E1]), six androgens (11-keto-testosterone [11-K], androstenedione [ADSD], *cis*-androsterone [CAND]), dihydrotestosterone [DHT], epitesosterone [EPI], and testosterone [TES]). The two sterols (3-beta-coprostanol [COP] and cholesterol [CHO]) and bisphenol A (BPA), along with six compounds noted below that are determined by the OWC method, are referred to as the Wastewater Micropollutants (WMPs). Information on these compounds can be found in Table S2. These compounds were included in this paper because their detection frequency was sufficient for computation of CSO bypass flow loads, and most of these compounds had sufficient detection frequency to compute treated plant effluent loads. Details of criteria used to determine if a compound could have loads computed are given in the Load Estimation section.

Organic Wastewater Compound (OWC) Method

The OWC method is described in Zaugg and others (2006) and uses continuous liquid-liquid extraction with methylene chloride solvent on unfiltered water samples. This method uses gas chromatography/mass spectrometry operated in full scan mode for determination of 69 OWCs. Results of six analytes (beta-sitosterol [SIT], benzophenone [BEP], caffeine [CAF], galaxolide [GAL], tris(2-butoxyethyl)phosphate [TBEP], and triclosan [TCS]) from the OWC method are discussed in this paper; detection frequency of these compounds was sufficient to compute both CSO bypass loads and treated plant effluent loads (Table S2).

Quality Assurance Data

Hormone Method

Recoveries for hormone method compounds in laboratory spikes of reagent water prepared with each set of field samples for the period when these samples were analyzed are given in Table S2. The analytes were spiked into reagent water samples at 25 ng/L (assuming 0.5 L volume), except for BPA (250 ng/L) and CHO and COP (2500 ng/L). Mean recoveries for the estrogen and androgens discussed in this paper are between 90–115%, with RSD (relative standard deviations) from 10–18%. Mean recoveries for BPA were 126% (26% RSD). Mean recoveries for CHO and COP in these reagent-water spikes were higher (about 168%) as were the RSDs (about 38%), and are biased high for some of these spikes because of a small amount of GC injection carryover from proceeding injections of influent samples that had very high sterol concentrations. This minimal carryover was magnified in resultant determined concentrations (and, thus, recoveries) in these reagent-water spikes because of unusually low (<10%) cholesterol-d7 recoveries in unsalted reagent-water matrices only (unusually low cholesterol-d7 recoveries occurred in lab

spike and lab and field blank samples that were prepared using unsalted reagent water as discussed in Foreman and others, 2012). Mean cholesterol-d7 recoveries were substantially higher in field samples (58%; 32% RSD), and within the expected range of those in salted reagent-water matrices and comparable complex field-sample matrices (see tables 17–20 in Foreman and others, 2012). As such, CHO and COP concentrations in field samples likely are not high biased. As expected, absolute recoveries for isotopically labeled analogs of the hormone compounds (E1, E2, E3, ADSD, DHT, and TES) and BPA in the lab spikes were somewhat lower (mean ranging from 74-97%), and RSDs were higher (26-40%), compared to the analyte recoveries that are corrected by using the isotope dilution quantitation procedure.

Recoveries in treated effluent spikes using two bottle types were within similar ranges, and compared well with reagent-water spikes, except that the recoveries for BPA, and, especially, CHO and COP had substantial bias because of high concentrations in the corresponding unspiked samples compared to the fortification concentration.

For the 12 lab reagent-water blanks analyzed using the hormone method, there was one detection below the reporting level for ADSD, E1, E2, and DHT, and one ADSD detection above the reporting level at 3.4 ng/L. There were four COP and five CHO detections above the reporting level up to 3600 ng/L in these blanks. The exception was one lab blank that followed an influent sample that had COP and CHO detections near 10,500 ng/L; these high concentrations were attributed to carryover during GC/MS/MS, and the resultant reported COP and CHO concentrations in this and other lab (and field) blanks were further biased high because of unusually low cholesterol-d7 recoveries in reagent-water matrices only, as discussed above and in Foreman and others (2012). Thus, these lab blank concentrations would represent a substantial overestimation of possible blank or instrumental carryover contributions to reported field sample concentrations for CHO and COP. Of the 23 field blanks (prepared using reagentwater) collected over the study and analyzed for hormone analytes, only three analytes were detected: one blank detection for ADSD at 1.5 ng/L, two blanks for COP (<2600 ng/L) and four blanks for CHO (<6000 ng/L). With the exception of ADSD, these blanks were less than any environmental concentration reported in this study. The one blank detection for ADSD was within half of the reporting level for ADSD; thus, all reported ADSD concentrations less than the RL were censored to insure that blank contamination would not affect interpretation of data. All concentrations in field samples of COP less than 5000 ng/L and concentrations of CHO less than 12,000 ng/L were censored. These censor thresholds were considered adequate to protect against positive bias in reported concentrations based on the overestimation of COP and CHO concentrations in reagent-water blanks because of poor cholesterol-d7 recoveries in those blanks. Blank data were not subtracted from field sample data for the hormone or OWC methods. Twenty eight (28) replicates were analyzed using for the hormone compounds. There were 163 comparisons with detections reported in each sample, and 7 with a detection reported in only one of the two replicates. The median relative percent difference (RPD) was 14% for these comparisons, and most comparisons (75%) had RPDs <30%.

Organic Wastewater Compound (OWC) Method

The six OWC compounds had median spike recoveries of 80% and median RSD (relative standard deviations) of 8.5% for reagent, surface and ground water spikes (Zaugg and others, 2006). Average recoveries for these spikes ranged from 62-105%, with RSDs between 5 and 16%. Recoveries for beta-sitosterol were generally lower (median of 55%) and more variable (median RSD =22%) than the other five compounds reported for the OWC method. Seven field blanks collected over the course of the study were analyzed using the OWC method. These blanks included a variety of field and processing equipment blanks, including auto-sampling equipment, bottles, and filters. None of the six OWC compounds included in this study had a detection reported in blank samples. Six replicate samples were analyzed for OWC compounds; 28 comparisons had a detection reported in both samples, 4 had a detection reported in only one sample, and the remainder (4) had no reported detection in both samples. The median of RPDs for OWC replicate comparisons was 13%, and most (75%) had differences less than 40%.

CALCULATIONS

Removal Efficiencies

Removal efficiencies between plant influent and plant effluent concentrations were calculated for the 17 paired PI and PE samples using the following formula: Where C_{PI} = concentration in PI sample and C_{PE} = concentration in PE sample. In instances when a concentration is a non-detection, a value of ½ of the reporting level is used to estimate the concentration for computing purposes. The estimates using ½ of the reporting level probably represent a conservative (low) estimate of removal efficiency, as the actual concentration could be much less.

Load Estimation

Continuous flow data from the CSE and PE sites are based on data collected at the Main Burlington WWTP. Overall flow rates for composite samples were calculated by averaging the flow over the time period corresponding to sample collection for all sample types. CSO bypass flows are equal to the sum of flow at the CSE and PE sites over the sample's collection period. Plant effluent sample flow was equal to the flow measured at the PE site over the sample collection period. Loads were calculated using a rating approach whereby a concentrationdischarge relation was calculated using the tobit regression approach discussed in Helsel and Hirsch (2002). The tobit regression technique is appropriate when regression is performed on variables with a high amount of censoring (>20%), and uses a maximum-likelihood estimation to derive coefficients. Both compound concentrations and discharge values were log-transformed, and the Duan smearing estimator was used to account for log-transformation bias (Helsel and Hirch, 2002). Regression statistics and load estimates are provided in Tables S4a-S4b. Loads were estimated based on an annual basis using flow data collected from December 1, 2007 to November 30, 2008. During this period, daily treated plant effluent flows ranged from 120 to 520 L/s, with an average of 200 L/s; there were 37 days with CSO bypass discharges, with these CSO bypass flows ranging from 550 to 2900 L/S, and an average duration of 3.3 hours. CSO bypass flows occurred in response to rainfalls ranging from 0.2 cm to 4.1 cm; snowmelt also contributed to winter bypass flows. CSO bypass flow constituted 10% of the total flow (CSO bypass flow and treated plant effluent discharges combined) during this period. (Note that the data collection period of November 2007-December 2008 includes 14 months; however, flows during the 12 month period of December 2007-November 2008 period were used as a basis for computing annual loads).

Treated plant effluent loads are based on concentrations in PE samples, and include 20 samples for compounds determined using the hormone method and 22 for compounds determined using the OWC method. The only exception to this is that 19 samples were used to determine treated plant effluent loads for ADSD and CAND, due to elevated detection limits for these two compounds in three samples. The range in flows for the PE samples was from 139 to 539 L/S, which is similar to the range in daily flows for treated plant effluent during the load estimation period (120 to 520 L/s). CSO bypass loads are based on all of the CSE samples collected over the course of the study as well as PI samples collected at discharges greater than 260 L/s. Samples collected at CSE range from 1400 L/s to 3240 L/s; however, discharges for the 39 CSO bypass flow events ranged from 550 to 2900 L/s. Examination of concentration-discharge curves (Figures S2a,S2b) indicated that significant dilution of many compound concentrations occurred in the CSE and PI samples at around 260 L/s. Thus, inclusion of PI samples collected at flows >260 L/s is important to adequately characterize the full range of the concentration-discharge relation for CSO bypass flow discharges. The number of samples to determine the CSO bypass loads for compounds determined by the hormone method was 21 samples, and 23 samples were used to determine the CSO bypass loads for compounds determined by the OWC method.

Helsel and Hirsch (2002) note that one of the drawbacks to the tobit regression approach is that it can be strongly influenced by outliers in the data set. To test whether outliers could affect load estimate for select compounds, loads also were calculated for all compounds using the Kendall-Theil robust line program given by Granato (2006) provided at

http://pubs.usgs.gov/tm/2006/tm4a7/. The Kendal-Theil slope is the median of all slopes that can be calculated between each datapoint in the data set. The use of this non-parametric method was motivated by the non-normal distribution of the concentration data for treated and untreated samples, and this approach ensures that outliers within the data set do not bias the slope of the concentration-discharge line. This robust method generates a regression that does not depend on a normal distribution of residuals, as required by ordinary least squares regression (Helsel and Hirsch, 2002). This method uses the Duan smearing estimator to insure that the rating curve estimation for loads is not biased by log-transformation.

Treated plant effluent load estimates by the Kendall-Theil robust line method were generally lower than those estimated by the tobit regression line (Table S5). The median of Plant Effluent loads calculated using Kendall-Theil regression method are 82% of the load calculated using tobit regression. CSO bypass flow load estimates by the Kendall-Theil robust line method, however, were somewhat higher than those estimated by the tobit regression line. The median of CSO bypass loads calculated using Kendall-Theil regression method are 123% of the load calculated using tobit regression (Table S5).

Overall, use of the Kendall-Theil robust line load estimates would not have affected the interpretations of the importance of CSO bypass versus treated plant effluent loads. With the exception of E3 (21%), the absolute difference between the percent of total load as CSO bypass flow load for the two methods was <10%. For E3, the proportion of total annual load from CSO bypass flow is 49% for the tobit estimate, and 70% for the Kendall-Theil estimate. By contrast, for E1 the tobit estimate of proportion of annual load from CSO bypass is 7%, whereas the Kendall-Theil estimate is 9%. The relatively high difference for E3 is probably due to the low proportion of detections for E3 in PE samples (55%). Because the tobit method is considered a better choice for determining regression equations for highly censored data, the tobit estimate is more appropriate for E3 than the load estimate by the Kendall-Theil robust line. Despite these differences, the conclusion that a substantial portion of the E3 load is transported by CSO bypass flows and that a small proportion of the E1 load is from CSO bypass flows is supported by both load estimate techniques. These results reveal that although the quantities of the load estimates are affected by the choice of statistical model used to estimate the concentration-discharge relation, the relative ratio of CSO bypass flow loads to treated plant effluent loads are not greatly affected by the choice of statistical technique used to estimate loads.

RESULTS

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A variety of figures and tables are included that give additional details that support the interpretations in the main body of the article. Because the space limitations in the journal article allowed for limited plots, plots for all the data are given in the section below. The figures and tables are listed individually below to guide the reader through this material.

Figure S2a shows the concentration-discharge relations for estrogens and androgens for samples used to compute the CSO bypass loads and the samples used to compute the treated plant effluent loads. Figure S2b shows the concentration-discharge relations for WMPs for samples used to compute the CSO bypass loads and the samples used to compute the treated plant effluent loads.

Figure S3 gives the range in concentrations for total suspended solids for samples used to compute the CSO bypass loads and the samples used to compute the treated plant effluent loads.

Figure S4a shows the annual loads and the annual loads plus twice the standard error and load estimate minus twice the standard error for estrogens and androgens for CSO bypass loads and the treated plant effluent loads. Figure S4b shows the annual loads and the annual loads plus twice the standard error and load estimate minus twice the standard error for WMPs for CSO bypass loads and treated plant effluent loads.

Figure S5 gives the percent of total annual load (which is equal to the CSO bypass load plus treated plant effluent load) from the combined sewer overflow. In addition, the range in removal for each of the target analytes is provided, with analytes arranged in increasing median removal along the x-axis.

Figure S6a shows plots of the percent decrease in concentration from the PI to PE samples as a function of effluent discharge for selected estrogens and androgens. Figure S6b shows plots of the percent decrease in concentration from the PI to PE samples as a function of effluent discharge for WMPs.

Figure S7 shows bar charts of the percent of annual total treated plant effluent load from the days with treated plant effluent flows greater than the plant design capacity (325 liters per second).

Figure S8a shows the range in ratios of concentrations of CAF to BEP, GAL, and TBEP, and ratios of ADSD to BEP, GAL, and TBEP, and ratios of CAND to BEP, GAL, and TBEP. Figure S8b shows the range in ratios of COP to BEP, GAL, and TBEP, and the range in ratios of CHO to BEP, GAL, and TBEP. These plots are in support of the discussion in the section below titled 'Assessment of Similarity between PI and CSE Samples'.

Several tables are included to support the conclusions and interpretations in the main body of the paper.

Table S1 gives details of the samples collected from the three sample collection points during the course of the study.

A list of compounds with sufficient detection frequency to compute loads for untreated samples are in Table S2; information includes the abbreviation used in the text for the compound, the analytical method used to determine concentration, the compound use or type, the CAS number of the compound, the log Kow of the compound, the reporting level for the compound, and percent detection for samples used to determine plant effluent load and combined sewer overflow load.

Table S3 gives details on method performance for the hormone method.

Table S4a provides the statistics for the tobit regression for concentration-discharge relation for hormones and wastewater micropollutants and annual load estimate for plant effluent discharge. Table S4b provides the statistics for the tobit regression for concentration-discharge relation for hormones and wastewater micropollutants and annual load estimate for combined sewer overflow discharges

Table S5 provides a comparison of annual loads calculated using tobit regression with those using the Kendall-Theil regression for both plant effluent loads and combined sewer overflow loads.

Assessment of Similarity between PI and CSE Samples

Load estimates for bypass flow are estimated based on Plant Influent (PI) samples collected at flows >260 L/s and all of the bypass flow effluent (CSE) samples collected during the study. Neither of these samples receives any biologic treatment. The PI samples were collected after the grit chamber, and the CSE samples were collected after vortex separation, so physical treatment for both samples were minimal. Although a small amount of water can be stored before vortex separation, once this storage is satisfied, water flows through the bypass flow portion of the plant, so retention time is minimal when CSO bypass flow occurs. A previous study (Muller and others, 2008) has indicated that primary treatment has little effect on hormone removal, suggesting that the CSE and PI samples reflect untreated concentrations of the compounds included in this study.

To test this assumption, an analysis of ratios of concentrations of compounds that are efficiently removed to ratios of concentrations of compounds that are poorly removed was performed among all sample types (PI, CSE, and PE). Efficiently removed compounds include those compounds with median removals >90%, and poorly removed compounds include those compounds with median removals <90%.

A similar analysis based on ratios of efficiently removed to poorly removed compounds was used by Kahle and others (2009). Ratios of efficiently removed to poorly removed concentrations should be greater in untreated water than in treated wastewater due to the greater degree of removal of efficiently removed compounds in treated wastewater, and ratios for PI and CSE samples should be similar, and both should be different than ratios for PE samples. This approach was used instead of simple comparison of concentrations among different sample types due to the demonstrated decrease in concentrations of compounds with increasing flow for the PI and CSE samples.

For this analysis, ratios of five efficiently removed compounds (CAF, ADSD, CAND, COP and CHO) were compared to concentrations of three poorly removed compounds (BEP, GAL, and TBEP) (see Figures S8a, S8b) for the same samples. The ratio of efficiently removed compound to poorly removed compound concentrations are greater for PI and CSE samples than for PE samples, indicating that the PE samples have higher relative concentrations of poorly removed compounds than efficiently removed compounds. The comparison indicates that the ratios of concentrations of efficiently removed to poorly removed compounds are generally similar between PI and CSE samples. Of the 15 ratios presented in Figures S8a and S8b, nine ratios have no significant difference between PI and CSE samples, five have higher ratios for PI samples cocurred for ratios of ADSD to poorly removed compounds; all of the higher ratios for CSE compared to PI were for ratios of CAND to poorly removed compounds. These results indicate that the assumption that PI and CSE both represent waters unaffected by treatment processes is reasonable, and that it is reasonable to pool CSE and PI data

to calculate loads. The similarity of the CSE and PI ratios also indicates that bromination of CSE samples for disinfection did not appear to have any important effect on concentrations.

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Figure S1. Schematic diagram of main Burlington Vermont wastewater treatment plant showing sampling locations for plant influent (PI), combined sewer effluent (CSE), and plant effluent (PE).



Figure S2a. Concentration as a function of discharge for hormones in samples collected from Main Burlington wastewater treatment plant, November 2007 to December 2008. These lines are tobit regression line used to determine loads from combined sewer overflow bypass and treated plant effluent discharges. For all analytes except epitestosterone, non-detected values are plotted along the x-axis, which is 1/2 of the reporting level concentration. For epitestosterone, non-detects are plotted at 2 ng/L, which represents 1/2 of reporting level. na denotes no correlation determined due to low number of detections.



Figure S2b. Concentration as a function of discharge for wastewater micropollutants in samples collected from Main Burlington wastewater treatment plant, November 2007 to December 2008. These lines are tobit regression line used to determine loads from combined sewer overflow bypass and treated plant effluent discharges. For all analytes but benzophenone and caffeine, non-detects are plotted along the x-axis, which represents 1/2 of the reporting level. For benzophenone and caffeine, non-detections are plotted at 100 ng/L. na denotes no correlation determined due to low number of detections.



Figure S3. Concentration as a function of discharge for total suspended solids (TSS) in samples collected from Main Burlington wastewater treatment plant, November 2007 to December 2008. These lines are tobit regression line used to determine loads from combined sewer overflow bypass and treated plant effluent discharges.



Figure S4a. Annual hormone loads for combined sewer overflow bypass and treated plant effluent discharges from Main Burlington wastewater treatment plant for period December 2007-November 2008. Loads estimated from concentration-discharge relation derived using tobit regression.



Figure S4b. Annual wastewater micropollutant loads for combined sewer overflow bypass and treated plant effluent discharges from Main Burlington wastewater treatment plant for period December 2007-November 2008. Loads estimated from concentration-discharge relation derived using tobit regression.



Figure S5. A) Percent of annual total load from combined sewer overflow bypass discharges and B) range in removal efficiency for samples collected from Main Burlington wastewater treatment plant, 2007-2008. Loads are based on tobit regression. Removal efficiency is the percent change from plant influent to plant effluent samples.



Figure S-6a. Removal efficiency with effluent discharge for hormones for samples collected at Main Burlington wastewater treatment plant, November 2007-December 2008.



Figure S-6b. Removal efficiency with effluent discharge for wastewater micropollutants for samples collected at Main Burlington wastewater treatment plant, November 2007-December 2008.



Figure S7. Percent of annual treated plant effluent load from days with plant effluent flow greater than design capacity (325 L/s) for indicated compounds. Approximately 10% of annual plant effluent flow occurs with days when flow is greater than design capacity.



Figure S8a. Ratios of concentrations of indicated compounds by sample type.

Ratios include caffeine to benzophenone, galaxolide and TBEP; androstenedione to benzophenone, galaxolide, and TBEP; cis-androsterone to benzophenone, galaxolide, and TBEP. TBEP=tris(2-butoxyethyl)phosphate.



Figure S8b. Ratios of concentrations of indicated compounds by sample type. Ratios include 3-beta-coprostanol to benzophenone, galaxolide and TBEP, and cholesterol to benzophenone, galaxolide, and TBEP. TBEP=tris(2-butoxyethyl)phosphate.

Table S1. Site code, date, time, sample collection method, number of composites, flow data and analytical method used for samples collected during CSO study. [PE=Plant Effluent; PI=Plant Influent; CSE=combined sewer effluent; GRA=grab sample; EWI=Equal Width Increment; FWC=Flow weighted composite; MSC, multi-sample composite, not flow weighted; na, not applicable; H=Hormone method (Foreman and others, 2012); W=Organic wastewater compound (Zaugg and others, 2006)].

Site Code	Date	Time	Sample Collection Method	Number of 1 Liter Composites Collected	Time Between 1-L Samples, in hours	Flow, liters/sec	Analytical Method
PE	11/20/2007	0100	FWC	24	1	178	W
PE	11/22/2007	0910	FWC	24	1	460	W
PE	11/27/2007	1400	FWC	12	1	287	H,W
PE	1/11/2008	2200	FWC	24	1	402	H,W
PE	3/9/2008	0230	FWC	12	1	332	H,W
PE	3/19/2008	1400	FWC	24	1	462	H,W
PE	3/26/2008	0010	FWC	24	1	262	H,W
PE	4/12/2008	0510	FWC	24	1	387	H,W
PE	4/29/2008	1900	FWC	24	1	285	H,W
PE	6/4/2008	2330	FWC	24	1	139	H,W
PE	6/10/2008	1900	FWC	10	1	329	H,W
PE	6/11/2008	0630	FWC	14	1	158	H,W
PE	7/14/2008	0630	FWC	24	1	300	H,W
PE	7/24/2008	1100	FWC	24	1	315	H,W
PE	8/12/2008	2300	FWC	24	1	213	H,W
PE	9/3/2008	2330	FWC	24	1	173	H,W
PE	10/25/2008	2310	FWC	8	1	539	H,W
PE	10/26/2008	1230	FWC	16	1	243	H,W
PE	11/5/2008	0100	FWC	24	1	146	H,W
PE	12/10/2008	1240	FWC	8	1	347	H,W
PE	12/11/2008	0140	FWC	16	1	167	H,W
PE	12/11/2008	1300	FWC	8	1	145	H,W
PI	11/19/2007	2000	FWC	24	1	178	W
PI	11/22/2007	0840	FWC	24	1	560	W
PI	11/27/2007	0130	FWC	12	1	819	H,W
PI	11/27/2007	1330	FWC	12	1	287	H,W
PI	1/11/2008	2130	FWC	24	1	487	H,W
PI	3/9/2008	0200	FWC	12	1	1010	H,W
PI	3/9/2008	0800	FWC	12	1	332	H,W
PI	3/19/2008	1830	FWC	24	1	773	H,W

ΡI	3/25/2008	1800	FWC	24	1	262	H,W
ΡI	4/12/2008	0440	FWC	24	1	663	H,W
ΡI	6/4/2008	1830	FWC	24	1	139	H,W
ΡI	6/10/2008	1830	FWC	10	1	598	H,W
ΡI	6/11/2008	0600	FWC	14	1	158	H,W
ΡI	7/14/2008	0600	FWC	24	1	574	H,W
ΡI	7/24/2008	1000	FWC	21	1	612	H,W
ΡI	8/12/2008	1800	FWC	24	1	213	H,W
ΡI	9/3/2008	1830	FWC	24	1	173	H <i>,</i> W
ΡI	10/25/2008	2240	FWC	8	1	1860	H <i>,</i> W
ΡI	10/26/2008	1200	FWC	16	1	243	H <i>,</i> W
ΡI	11/4/2008	2000	FWC	24	1	146	H <i>,</i> W
ΡI	12/10/2008	2000	FWC	24	1	231	H <i>,</i> W
CSE	11/22/2007	0100	GRA	na	na	1530	W
CSE	11/26/2007	2345	MSC	6	0.5	1780	H <i>,</i> W
CSE	1/11/2008	1600	FWC	6	0.25	1400	H <i>,</i> W
CSE	3/8/2008	2100	FWC	11	0.5	1550	H <i>,</i> W
CSE	3/20/2008	0100	FWC	12	0.5	2280	H,W
CSE	4/12/2008	0430	MSC	6	0.5	2620	H,W
CSE	4/29/2008	0900	FWC	5	0.5	1950	H <i>,</i> W
CSE	6/10/2008	2010	FWC	5	0.33	1920	H,W
CSE	7/13/2008	1800	FWC	15	0.25	1830	H,W
CSE	7/23/2008	2100	FWC	14	0.25	3240	H,W

Table S2. Compounds with sufficient detection frequency to compute combined sewer effluent loads for Main Burlington wastewater treatment plant, 2007-2008. [Samples used to compute Combined sewer overflow bypass loads include plant influent samples collected above a discharge of 260 L/s and all combined sewer effluent samples. Plant effluent samples include all plant effluent samples collected during the study. H=Hormone method (Foreman and others, 2011); OWC=Organic wastewater compound (Zaugg and others, 2006)].

Percent Detection

Compound	Abbrevi							Combine			
·	ation	Me-	Compound			_	Plant	d Sewer			
		thod	Use/Type	CAS	Log Kow	RL ^a	Effluent	Overflow			
Hormones											
Estrogens											
17-beta-estradiol	E2	н	Estrogen	50-28-2	4.01 ^b 2.45.	0.8	25	93			
Estriol	E3	Н	Estrogen	50-27-1	3.67 ^b	0.8	55	100			
Estrone	E1	н	Estrogen	53-16-7	3.13 ^b	0.8	90	100			
Androgens											
11-Ketotestosterone	11-K	Н	Androgen	564-35-2	1.92 ^c	0.8	0	75			
Androstenedione	ADSD	Н	Androgen	63-05-2	2.75 ^b	2	84	100			
<i>cis</i> -Androsterone	CAN	Н	Androgen	53-41-8	3.69 ^b	0.8	74	100			
Dihydrotestosterone	DHT	н	Androgen	521-18-6	3.55 ^b	4	5	96			
Epi-testosterone	EPI	н	Androgen	481-30-1	3.32 ^b	4	0	76			
Testosterone	TES	Н	Androgen	58-22-0	3.32 ^b	0.8	5	96			
			Wastewater M	icropollutants	;						
3-beta-Coprostanol	СОР	н	Sterol	360-68-9	8.82 ^b	2000	95	100			
Benzophenone	BEP	OWC	UV Absorber	119-61-9	3.15	200	86	83			
beta-Sitosterol	SIT	OWC	Plant Sterol Plastic	83-46-5	9.65	800	86	100			
Bisphenol A	BPA	Н	component	80-05-7	3.32 ^d	100	53	60			
Caffeine, wu ug/l	CAF	OWC	Beverages	58-08-2	0.16	200	64	100			
Cholesterol	СНО	н	Steol	57-885	8.74 ^b	2000	90	100			
Galaxolide	GAL	OWC	Musk Fragrance	1222-05-5	6.26	200	100	100			
Tri(2-butoxyethyl) phosphate (TBEP)	TBE	owc	Plasticizer	78-51-3	3.00	200	95	100			
Triclosan	TCS	OWC	Antimicrobial	3380-34-5	4.66	200	100	100			

^aRL = Reporting level; ^b experimental value compiled by Yang et al. (2011) Supplemental

Information (SI); ^c value from KOWWIN computer model compiled by Yang et al. (2011) SI;

^d experimental value from Hansch et al. (2005)

Table S3. Percent recoveries for reagent laboratory spikes analyzed between November 2007 and December 2008 and spikes of treated effluent in a plastic (HDPE) and in a Teflon bottle for hormone method compounds and the isotope dilution standards available for these compounds. [Fortification level was 25 nanograms per liter (ng/L) for the steroid hormones, 250 ng/L for bisphenol A, and 2,500 ng/L for 3-beta-coprostanol and cholesterol assuming a 0.5-L sample volume. Isotope dilution standards were fortified at 100 ng/L, except cholesterol-d7, which was fortified at 10,000 ng/L. HDPE=High density polyethelene.]

	Laborat	ory Reagent W	/ater Spikes	Treated Effluent HDPE Bottle Spike	Treated Effluent Teflon Bottle Spike	
Parameter	Number of Spikes	Mean Recovery, in Percent	Relative Standard Deviation, in Percent	Recovery, in Percent	Recovery, in Percent	
Estrogens						
17-beta-Estradiol	12	103	11	99	97	
Estriol	12	109	12	119	124	
Estrone	12	113	18	105	113	
Androgens						
11-Ketotestosterone	12	105	15	89	90	
4-Androstene-3,17- dione	12	105	15	96	97	
cis-Androsterone	12	101	10	141 ^a	156 [°]	
Dihydrotestosterone	12	89	12	96	100	
Epitestosterone	12	104	11	108	112	
Testosterone	12	107	19	100	103	
Other Compounds						
3-beta-Coprostanol	11	168 ^b	36	244 ^d	175 [°]	
Bisphenol A	9	119	23	218^d	76 ^d	
Cholesterol	11	169 ^b	38	179 ^c	103 ^c	
Isotope Dilution Standards						
17-beta-Estradiol-d4	12	78	19	73	71	
4-Androstene-3,17- dione-2,2,4,6,6,16,16- d7	12	77	40	75	72	
Dihydrotestosterone- 1,2,4,5a-d4	12	93	28	36	32	
Estriol-2,4,17-d3	12	85	29	78	75	
Estrone-2,4,16,16-d4	12	74	28	76	72	
Testosterone-2,2,4,6,6- d5	12	79	29	73	69	
Bisphenol-A-d16	9	77	20	109	117	

Cholesterol-d7	12	20	120	47	48

^a*cis*-Androsterone concentrations in the corresponding unfortified samples ranged to 44% of the fortification amount.

^bCholesterol and coprostanol concentrations in non-salted reagent-water matrices only, including these reagentwater spikes, are biased high largely because on unusually low (<10%) cholesterol-d7 recoveries in 8 of the 12 spikes (see Foreman and others, 2012). Mean cholesterol-d7 recoveries (58%; 32% RSD) were substantially higher in field samples and comparable to those in salted reagent water (Foreman and others, 2012).

^cThe analyte concentrations in the corresponding unfortified replicates exceed the fortification amount by >900%.

^dThe bisphenol A concentrations in the corresponding unfortified replicates exceed the fortification amount by >150%.

Table S4a. Statistics of tobit regression for concentration-discharge relation for indicated hormone and annual load estimate for treated plant effluent at Main Burlington Vermont wastewater plant, December 2007-November 2008. Regression is for base-10 log transformed concentration and base-10 log transformed discharge. Number represents the number of samples used in tobit regression, %Cens is the number of censored observations (non-detects), Int=Intercept for regression, Slope=Slope of regression, SES is the standard error of slope, SigSlope is the statistical significance of the slope, RMSE is the root mean square error, P-R2 is the pseudo R-squared, Duan is the Duan smearing estimator, Load is the annual load in grams, and HighLoad is the load corresponding to the 36 days with the highest treated flow, in grams.

		%C									
	Numb	en				Sig	RMS	P-	Dua		High
Compound	er	S	Int	Slope	SES	Slope	E	R2	n	Load	Load
Fatrogona					Hormo	nes					
Estrogens											
Estriol	20	45	-3.93	1.53	0.89	0.0849	0.763	0.162	2.32	7.14	1.8
Estrone	20	10	-1.732	0.996	0.57	0.0857	0.503	0.131	1.68	43.3	8.31
Androgens											
Androstene-											
dione	19	16	-0.541	0.383	0.294	0.193	0.335	0.082	1.12	16.1	2.18
cis-	10	22	F 022	2 505	0.021	0.00715	0.026	0 202	a aa	21.0	0.00
Androsterone	19	32	-5.832	2.505	0.931	0.00715	0.836	0.303	2.33	21.0	8.09
3-beta-			v	Vastewater	Micropoll	utants					
Coprostanol	20	5	1.12	1.25	0.384	0.0011	0.352	0.35	1.2	88500	19400
Benzophenone	22	14	0.533	-0.507	0.217	0.0198	0.21	0.2	1.09	1590	122
beta-Sitosterol	22	13	-2.46	1.08	0.366	0.00303	0.363	0.292	1.23	9400	1890
Bisphenol-A	15	47	1 215	0 334	0 233	0 308	0 261	0.076	1 73	773	97.6
Displicitor A	15	47	1.215	0.554	0.235	0.500	0.201	0.070	1.25	115	57.0
Caffeine	22	37	-7.76	2.96	0.821	0.00032	0.642	0.456	1.002	1420	642
Cholesterol	20	10	0.968	1.332	0.565	0.0184	0.631	0.220	1.37	110000	25600
Galaxolide	22	0	0.522	-0.226	0.13	0.0838	0.117	0.12	1.03	6530	603
TBEP	22	4.5	-2.259	1.039	0.701	0.138	0.641	0.091	2.72	9124	5170
Triclosan	22	0	-1.705	0.513	0.283	0.0699	0.253	0.13	1.14	2270	331

Table S4b. Statistics of tobit regression for concentration-discharge relation for samples used to estimate load of combined sewer overflow bypass loads for Main Burlington wastewater treatment plant, December 2007-November 2008. Regression is for base-10 log transformed concentration and base-10 log transformed discharge. Number represents the number of samples used in tobit regression, %Cens is the number of censored observations (non-detects), Int=Intercentp for regression, Slope=Slope of regression, SES is the standard error of slope, SigSlope is the statistical significance of the slope, RMSE is the root mean square error, P-R2 is the pseudo R-squared, Duan is the Duan smearing estimator, Load is the annual load in grams.

Compound	Number	%Cens	Int	Slope	SES	Sig Slope	RMSE	P-R2	Duan	Load	
Hormones											
Estrogens											
17-beta-estradiol	21	9.5	3.74	-1.13	0.151	0.0001	0.245	0.736	1.14	0.828	
Estriol	21	0	6.31	-1.64	0.147	0.0001	0.234	0.856	1.13	6.83	
Estrone	21	0	5.24	-1.4	0.122	0.0001	0.194	0.862	1.08	3.31	
Androgrens											
11-Ketotestosterone	21	24	7.28	-2.23	0.285	0.0001	0.466	0.763	1.49	1.07	
Androstenedione	21	0	6.35	-1.56	0.191	0.0001	0.304	0.76	1.18	14.2	
cis-Androsterone	21	0	3.619	-0.345	0.193	0.074	0.306	0.132	1.21	241	
Dihydrotestosterone	21	4.8	5.61	-1.46	0.207	0.0001	0.354	0.707	1.21	5.56	
Epi-testosterone	21	19	5.24	-1.47	0.151	0.0001	0.222	0.846	1.19	2.17	
Testosterone	21	0	4.8	-1.21	0.108	0.0001	0.172	0.855	1.08	4.96	
			Wast	ewater Mi	cropollut	ants					
3beta-Coprostanol	21	0	6.745	-0.535	0.196	0.00629	0.31	0.262	1.27	80800	
Benzophenone	23	17	1.078	-0.602	0.132	0.0001	0.225	0.49	1.1	91.1	
beta-Sitosterol	23	0	1.2332	-0.0202	0.181	0.911	0.291	0.0005	1.27	12100	
Bisphenol-A	15	40	4.46	-0.78	0.204	0.0001	0.253	0.563	1.29	67.6	
Caffeine, wu ug/l	23	0	4.077	-0.993	0.159	0.0001	0.257	0.628	1.13	4970	
Cholesterol	21	0	6.653	-0.506	0.176	0.00396	0.279	0.283	1.2	76900	
Galaxolide	23	0	1.412	-0.489	0.135	0.000306	0.218	0.362	1.13	472	
Tri(2-butoxyethyl)											
phosphate (TBEP)	23	0	2.601	-0.693	0.283	0.0144	0.456	0.207	1.57	2190	
Triclosan	23	0	2.384	-0.797	0.152	0.0001	0.245	0.543	1.14	441	

Table S5. Comparison of annual load estimates for hormones and wastewater micropollutants using Tobit and Kendall-Theil load methods for A. treated plant effluent loads and B. combined sewer overflow bypass loads for Main Burlington wastewater treatment plant, December 2007-November 2008.

A. Treated Plant Effluent Loads			
Compound	Tobit Load (Grams)	Kendall-Theil Load (Grams)	Percent of Tobit load represented by Kendall- Theil Load
	Hormones		
Estrogens			
Estriol	7.14	4.12	58
Estrone	43.3	25.6	59
Androgens			
Androstenedione	16.1	14.2	88
cis-Androsterone	21.0	11.8	56
Waste	water Micropollu	utants	
3-beta-Coprostanol	88500	72300	82
Benzophenone	1590	1540	97
beta-Sitosterol	9400	7420	79
Bisphenol-A	774	670	87
Caffeine	1420	1590	112
Cholesterol	111000	89500	80
Galaxolide	6530	6900	106
ТВЕР	26400	13400	51
Triclosan	2270	2240	99

A. Treated Plant Effluent Loads

B. Combined Sewer Overflow Bypass

	Hormones		
Estrogens			
17-beta-estradiol	0.828	1.04	126
Estriol	6.83	9.8	143
Estrone	3.31	4.76	144
Androgens			
11-Ketotestosterone	1.07	1.66	155
Androstenedione	14.2	17.5	123
cis-Androsterone	241	295	122
Dihydrotestosterone	5.56	7.12	128

Epi-testosterone	2.17	2.87	132
Testosterone	4.96	5.57	112
	Wastewater Micropollu	tants	
3-beta-Coprostanol	80800	73300	91
Benzophenone	91.1	104	114
beta-Sitosterol	12100	13100	108
Bisphenol-A	67.6	97.6	144
Cholesterol	76900	72600	94
Caffeine	4970	5510	111
Galaxolide	472	521	110
TBEP	2190	1120	51
Triclosan	441	441	100