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Occurrence and Mass Loading of Synthetic Opioids, Synthetic Cathinones, and Synthetic Cannabinoids in Wastewater Treatment Plants in Four U.S. Communities

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Abstract

A few new psychoactive substances (NPS) that mimic the effects of controlled neuropsychiatric and illicit drugs have been forensically identified in the U.S. Wastewater-based epidemiology (WBE) can provide a comprehensive and more cost- and time-effective method of determining the prevalence of NPSs in communities. In this study, an analytical method capable of simultaneous determination of trace-level 40 NPS residues (synthetic opioids, synthetic cannabinoids, synthetic cathinones, piperazines, indole, and amphetamine) in wastewater was developed and validated. The developed analytical method was utilized to determine the occurrence of NPSs in four rural communities in southern Illinois. Nine NPSs (carfentanil, furanyl fentanyl, methoxyacetyl fentanyl, MAB-CHMINACA, methcathinone, 4-methyl pentedrone, 2-methyl-4′-(methylthio)-2-morpholinopropiophenone (MMMP), 1-(3-chlorophenyl) piperazine (mCPP), and 5-(2-Aminopropyl) Indole (5IT) were quantified. Methcathinone was the most frequently detected NPS (detection frequency, df = 100%) followed closely by the MMMP and mCPP (df = 91%). The mass loading of methcathinone, mCPP, and 5-IT using ammoniacal nitrogen-based population were up to 21.1 ± 1.3 mg/d/1000 people, 15.0 ± 0.5 mg/d/1000 people, and 9.75 ± 2.72 mg/d/1000 people, respectively. This is the first study to determine the occurrence of NPSs including synthetic opioids, synthetic cannabinoids, synthetic cathinones, and piperazines in the U.S. communities.

Graphical Abstract

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Supporting Information
TThe Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c00250.
Mobile phase compositions and programming, optimized MS/MS transitions and collision energies of target drugs, chromatograms showing drugs' chromatographic separation, optimized extraction efficiency, and the prevalnce of gabapentin in target communities (PDF)

The authors declare no competing financial interest.

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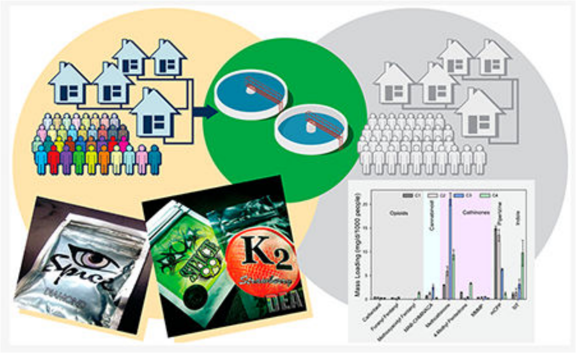

INTRODUCTION

New narcotics or psychoactive substances (NPS) in "pure" or "preparation" forms are created to have the similar effects of established prescribed and illicit neuropsychiatric drugs.[1] There were ~500 NPS in the global market each year from 2015 to 2017.[2] In the U.S., the forensic identifications of NPS tripled from 684 identifications in 2016 to 2023 identifications including 1783 opioids/analgesics, 81 synthetic cannabinoids, 55 synthetic cathinones, 46 benzodiazepines, and 58 "others" in 2019.[3] There were five NPS (carfentanil, furanyl fentanyl, 5-fluoro AMB, 5-fluoro ADB, and *N*-ethylpentylone) among 25 most prevalent drugs in the Midwest in 2017.[4] National Drug Early Warning System (NDEWS) reported 3099 counts of fentanyl, 670 counts of synthetic cathinones, and 633 counts of synthetic cannabinoids seizures in 2017 in Illinois by the law enforcement.[5]

The NDEWS has reported the prevalence of NPS based on the forensic analyses, which can underestimate the actual amount of abused drugs and cause a delay between the outbreak of a drug and the intervention of law enforcement. Wastewater-based epidemiology (WBE) provides a non-invasive, comprehensive, cost-effective, and near-real-time estimation of the prevalence of substance use in a community.[6–8] WBE has recently been demonstrated as a complementary tool to a forensic toxicological approach to comprehend a trend in the consumption of NPS in Australia.[9] NPSs are reintroduced in markets in quick succession to impede the law enforcement's efforts to control NPS production and purchases.[1] Therefore, the traditional approaches are not suitable for drug monitoring, surveillance, control, and the immediate responses to the harm associated with the NPS.[10,11] WBE can be used as a proactive public health surveillance system for NPS for prompt intervention. In WBE, the residual parent drugs or their metabolites in raw wastewater are utilized to back-calculate the prevalence of target drugs in a community.

NPS typically are more potent and toxic than established drugs of abuse, such as illicit drugs and prescribed opioids. Carfentanil, a synthetic opioid, is ~100 folds more potent than fentanyl and ~10 000 folds more powerful than morphine. Synthetic cannabinoids mimic the psychotropic effects of cannabis-derived cannabinoids (e.g., Δ^9-tetrahydrocannabinol: THC) binding similarly to the cannabinoid receptors; however, synthetic cannabinoids are more potent and efficacious cannabinoid receptors antagonists than THC.[12] Similarly, 3,4-*methylenedioxypyrovalerone*, a common synthetic cathinone, has similar effects as cocaine but ≥10 folds more powerful than cocaine.[1] Therefore, the potential ultratrace level of NPS in wastewater, a complex environmental matrix, poses analytical challenges for the detection and quantification. NPS in wastewater typically have been enriched using solid-phase extraction (SPE) and analyzed using a high-performance liquid chromatography tandem mass spectrometric technique (HPLC-MS/MS) for target quantification[13–15] as well as Orbitrap high resolution mass spectrometer[16,17] and time-of-flight mass spectrometer[18–20] for target and untargeted screening. The majority of the reports used either neutral HLB (hydrophilic–lipophilic balance) or mix mode cation-exchange cartridges (MCX or WCX) for the quantification of few NPSs of a particular class of drugs, such as synthetic cannabinoids or synthetic cathinones. Typically, nonacidified wastewater samples were extracted via the preconditioned HLB cartridge (with methanol followed by water) and eluted with methanol;[17,21] however, acidified (pH ~ 2–5) wastewater samples were extracted via the preconditioned MCX cartridge (with methanol followed by acidified water) and eluted with methanol followed by ammoniacal methanol (~2 or 5%).[16,17,22]

Bade et al.[23] found MCX-extraction more efficient than HLB-extraction for synthetic cathinones whereas Fontanals et al.[16] preferred WCX over MCX for synthetic cathinones. To the authors' knowledge, there was no report of the optimized extraction of synthetic fentanyls from wastewater. Salguero-Gonzalez et al.[16] used both HLB and MCX cartridges to cover the wide range of physio-chemical properties of NPSs including synthetic cathinones and synthetic cannabinoids. All WBE studies on NPS have focused on synthetic cannabinoids and synthetic cathinones. To our knowledge, there are no reports of simultaneous quantification of synthetic cathinones, synthetic cannabinoids, and synthetic opioids in wastewater. The prevalence of NPS in communities using WBE have been reported in Europe[11,13,17,24] Australia,[9,23] and China.[22] However, this is the first study of reporting NPSs in wastewater in the U.S.

In this study, an analytical method capable of simultaneous trace-level determination of 40 NPS residues including 11 synthetic cannabinoids, 10 synthetic cathinones, 15 synthetic opioids, 2 piperazines, 1 indole, and 1 amphetamine in wastewater has been developed and validated. The occurrence of NPS in four rural southern communities in Illinois was determined based on the level of NPSs measured for seven consecutive days. Moreover, clonazepam (benzodiazepine,) and gabapentin (anticonvulsant) were also determined as a potential chemical marker of NPS prevalence in target communities owing to the regional potential abuse.[25]

MATERIALS AND METHODS

Reagents and Chemicals.

Based on the frequent forensic identifications of NPS by the NDEWS in the U.S.,[3–6] 40 NPS were selected belonging to opioids/analgesics, synthetic cannabinoids, synthetic cathinones, piperazines, indoles, and amphetamines. Target drugs include opioids/analgesics: carfentanil, furanyl fentanyl, valeryl fentanyl, butyryl fentanyl, acetyl fentanyl, cyclopropyl fentanyl, benzyl fentanyl, 3′-methyl fentanyl, 4′-methyl acetyl fentanyl, 4-fluoro-isobutyryl fentanyl, para-fluorobutyryl fentanyl, methoxyacetyl fentanyl, 4-ANPP, U-48800, U-47700; synthetic cannabinoids: 5-fluoro MDMB-PICA, MAB-CHMINACA, MMB-FUBINACA, 5-

fluoro EDMB-PINACA, (R)-5-fluoro ADB, AB-FUBINACA, MMB-CHMICA, AB-CHMINACA, ADB-FUBINACA, 5-fluoro AMB, NM2201; synthetic cathinones: (±)-methcathinone, ethylone, 4-MMC/mephedrone (MMC), 4-methyl pentedrone, *N*-ethylpentylone, α-pyrrolidinopropiophenone, 3,4-methylenedioxypyrovalerone, α-ethylaminohexanophenone, 2-methyl-4′-(methylthio)-2-morpholinopropiophenone, 4-chloro-α-PVP; piperazines: 1-(3-chlorophenyl) piperazine (mCPP), 1-cyclohexyl-4-(1,2-diphenylethyl) piperazine (MT-45); indole: 5-(2-aminopropyl) indole (5IT); amphetamine: 4-methylamphetamine. Target drugs also include two of the most commonly used anticonvulsants in the U.S. gabapentin and clonazepam.

High purity standard solutions (50, 100, or 1000 μg/mL) or solids (1 or 5 mg) of each target drug and the corresponding deuterated internal standards (carfentanil-d_5, furanyl fentanyl-d_5, valeryl fentanyl-d_5, butyryl fentanyl-d_5, acetyl fentanyl-$^{13}C_6$, cyclopropyl fentanyl-d_5, 4-fluoro-isobutyryl fentanyl-d_7, 4′-methyl acetyl fentanyl-d_5, methoxyacetyl fentanyl-d_5, 4-ANPP-d_5, MAB-CHMINACA-d_4, U-48800-d_3, U-47700-d_3, AB-FUBINACA-d_4, *N*-ethylpentylone-d_5, methcathinone-d_3, ethylone-d_5, mephedrone-d_3, 3,4-methylenedioxypyrovalerone-d_8, mCPP-d_8, MT45-d_{11}, gabapentin-d_{10}, clonazepam-d_4) were purchased from Cerilliant (Round Rock, TX) or Cayman Chemical (Ann Arbor, MI) and stored at −20 °C. HPLC grade methanol and formic acid (99.5% purity) were purchased from Fisher Scientific (Hampton, NH), hydrochloric acid and ammonium hydroxide were purchased from Pharmco (Brookfield, CT), and ultrapure water was prepared with Barnstead Ultrapure System.

Sample Collection.

Samples from four WWTPs in four rural counties in Southern Illinois (C1, C2, C3, and C4) were collected for seven consecutive days during a typical week with no special events (to the best of our knowledge) in early August of 2019. An aliquot of untreated wastewater was withdrawn every 15 min using a time-proportional autosampler for 24 h, composited, and stored at 4 °C. However, grab samples were collected from C4 around noon due to the unavailability of an autosampler. The week-average wastewater inflow in C1, C2, C3, and C4 were 0.70 ± 0.10, 1.77 ± 0.38, 2.64 ± 0.19, and 0.47 ± 0.04 million gallons per day, respectively. All samples were collected in one-liter polypropylene bottles, transported on ice to the laboratory, stored at −20 °C, and extracted within 48 h.

Sample Preparation.

One hundred milliliters of acidified (HCl, pH ~ 2) raw wastewater in duplicates ($n = 2$) for each of the four sites were centrifuged at 4500 rpm for 5 min and filtered under vacuum using 0.45 μm white nylon filter paper to separate suspended particulate matter. The wastewater samples were spiked with 50 or 100 ng of internal standards, mixed well, and extracted using Oasis MCX 6 cc solid-phase extraction cartridge. Before extraction, cartridges were conditioned with 3.0 mL of methanol followed by 3.0 mL of acidified ultrapure water (formic acid, pH ~ 2). The samples were extracted at a rate of ~1 mL/min, dried under vacuum for ~5 min, eluted with 5.0 mL of 5% ammonia in methanol, and stored at −20 °C. The eluate was concentrated to ~250 μL under a gentle flow of nitrogen gas at ambient conditions. The concentrate was transferred quantitatively to an amber silanized LC vials and the final volume was adjusted to ~1.0 mL with methanol. One microliter of all prepared samples was subjected to the instrumental analysis.

Instrumental Analysis.

Ultrahigh-performance liquid chromatography (Agilent 1290 Infinity II LC System) was coupled with tandem mass spectrometry (Agilent 6460 triple quadrupole mass spectrometer) (Santa Clara, CA) to analyze the prepared samples for target drug residues. Target analytes were chromatographically separated (Figure 1, Supporting Information (SI) Figure S1) by using a Force Biphenyl column (100 mm× 2.1 mm i.d. × 1.8 μm particle size) and the gradient flow of HPLC-grade methanol and 0.1% aqueous solution of formic acid (SI Table S1). Relative retention time (±0.05 min) to their deuterated forms, two parent-to-daughter ion transitions (SI Table S2), and the ratio of the abundance of quantitative to qualitative ions (±20%) were used for target analyte peak identification. The collision energy for multiple reaction monitoring transitions were optimized for all analytes and internal standards (SI Table S2). The source parameters including gas temperature (330 °C), gas flow rate (5 L/min), nebulizer (30 psi), sheath gas temperature (250 °C), sheath gas flow rate (12 L/min), and capillary voltage (4000 V) were used.

Analytes were quantified using the isotopic dilution mass spectrometry method where a known quantity of deuterated isotopes of drugs was spiked into the sample prior to sample preparation. This method corrects for the loss of analytes during the sample preparation and instrumental analysis. However, closely related deuterated internal standards from the same class and or adjacent retention times were used for the quantification of select drugs that were not commercially available. Valeryl fentanyl-d_5 was used as an internal standard for 2-methyl-4′-(methylthio)-2-morpholinopropiophenone, cyclopropyl fentanyl-d_5 for 3′-methyl fentanyl, 4-fluoro-isobutyryl fentanyl-d_7 for para-fluorobutyryl fentanyl, 4′-methyl acetyl fentanyl-d_5 for benzyl fentanyl, methoxyacetyl fentanyl-d_5 for 4-chloro-α-PVP, MAB-CHMINACA-d_4 for 5-fluoro MDMB-PICA, MMB-FUBINACA, 5-fluoro EDMB-PINACA, (R)-5-fluoro ADB, MMB-CHMICA, AB-CHMINACA, 5-fluoro AMB, and NM2201, AB-FUBINACA-d_4 for ADB-FUBINA-CA, *N*-ethylpentylone-d_5 for 4-methyl pentedroneand α-ethylaminohexanophenone, methcathinone-d_3 for 5IT, and ethylone-d_5 for α-pyrrolidinopropiophenone and 4-methylamphetamine. The concentration-dependent response factor was plotted against the response-dependent concentration factor to produce a nine-to-12-point calibration curve for each target drug. The regression coefficients calculated by linear or quadratic regression were $r^2 \geq 0.99$ for all target analytes. All quantitate analysis was performed using Mass Hunter Version 10.0.

Optimization of Extraction Efficiency.

Oasis HLB (6 cc, 200 mg), MCX (6 cc, 150 mg), and WCX (6 cc, 150 mg) solid-phase extraction cartridges and different extraction eluents were evaluated for the optimum simultaneous extraction of target drugs. Hydrophilic–lipophilic balance (HLB) are broad-spectrum cartridges and ideal for acidic, basic, and neutral analytes. Mixed-mode, strong cation-exchange (MCX) cartridges are selective for bases with organic solvents, whereas weak cation-exchange (WCX) cartridges are mixed-mode and used for strong bases with organic solvents.[26] In this study, absolute spiking recoveries ($n = 3$) were determined at a lower spiking level (2.0 or 4.0 ng) as well as midpoint calibration level (50 or 100 ng).

The HLB cartridges were conditioned with 3.0 mL of methanol followed by 3.0 mL of ultrapure water to extract nonacidified (pH ~ 7) filtered wastewater. The extraction recovery of target drugs from HLB cartridges was also evaluated in acidic conditions (HLB-A), conditioned using 3.0 mL of methanol followed by 3.0 mL of acidified ultrapure water (formic acid, pH ~ 2) and extracted the acidified wastewater. All HLB cartridges were dried under vacuum for ~5 min, and eluted with 5.0 mL of 2% ammonia in methanol, as reported elsewhere.[21,24]

The MCX cartridges were conditioned with 3.0 mL of methanol followed by 3.0 mL of acidified ultrapure water (formic acid, pH ~ 2), used to extract ~100 mL of acidified wastewater, dried under vacuum, and eluted with 5.0 mL of 5% ammonia in methanol. However, WCX cartridges were conditioned with 3.0 mL of methanol followed by 3.0 mL of ultrapure water, extracted nonacidified (pH ~ 7) wastewater, dried under vacuum, and eluted with 5% formic acid in methanol, as described elsewhere.[15]

All eluates were collected and concentrated as described above, spiked with 50 or 100 ng of internal standards, and adjusted to a final volume of ~1 mL with methanol. The spiking recovery of drugs was also evaluated at more environmental relevant concentrations (spiking 2 or 4 ng of target analytes) similarly as described above. A control sample spiked with internal standard mixture (but not spiked with the target drugs) was analyzed at the beginning and the end of the analysis of sample batch; the average concentrations of drugs in the blank (if any) were subtracted from the samples. At 2 or 4 ppb spiking level, the absolute spiking recoveries of target NPSs using MCX and WCX ranged from 51.8 ± 0.34% (4-methylamphetamine) to 132 ± 3.2% (MAB-CHMINACA) and 48.0 ± 1.5% (4-methylamphetamine) to 128 ± 0.90% (cyclopropyl fentanyl), respectively, whereas the absolute spiking recoveries of target NPSs using HLB and HLB-A ranged from 50.6 ± 1.5% (4-methylamphetamine) to 115 ± 3.4% (MAB-CHMINACA) and 50.6 ± 4.0% (4-methylamphetamine) to 132 ± 2.5% (cyclopropyl fentanyl), respectively (Figure 2). Overall, the triplicate spiking recoveries of target NPSs using MCX extraction cartridge found an optimum at both spiking levels; MCX was considered for the extraction of NPSs from the collected wastewater samples to determine their occurrence in communities. More details are provided in the SI.

Quality Assurance and Quality Control.

Along with the analysis of sample extracts, a method blank and a matrix-spike sample ($n = 2$) were incorporated. A method blank consisted of acidified ultrapure water (pH ~ 2, HCl) spiked with 50 or 100 ng of internal standards prior to extraction as described above. A random wastewater sample was considered for matrix-spike samples, spiked with 50 or 100 ng of target analytes prior to extraction, and was processed as described above. As a calibration check standard, a mixture of standards at the midpoint calibration-level was analyzed after every 10 samples.

The method detection limit (MDL) was defined as the minimum concentration of an analyte that can be distinguished from the method blank and can be reported with 99% confidence.[27] MDL was performed according to the U.S. Environmental Protection Agency's Code of Federal Regulations.[27] Briefly, the wastewater samples (100 mL, $n = 7$) were acidified, centrifuged, filtered, and spiked with target drugs (0.1 or 0.2 ng) and internal standards (50 or 100 ng), and processed as described in Section 2.3. MDL was determined using the following equation:

$$\mathrm{MDL} = \mathrm{s} \times \mathrm{t}_{n-1,\,1-\alpha=0.99}$$

where s is the standard deviations of the measured concentrations of a drug in seven replicate samples and t is the Student's t at 99% confidence level (3.143 for n–1 = 6). The 95% confidence interval was estimated as

$$\text{lower confidence limit(LCL)} = 0.64 \times \mathrm{MDL}$$

$$\text{upper confidence limit(UCL)} = 2.20 \times \mathrm{MDL}$$

The limits of detection (LODs) and limits of quantification (LOQs) were defined as the calculated minimum concentration of analytes corresponding to the signal-to-noise ratio 3 and 10, respectively, in wastewater extracts ($n = 7$) spiked with standards at the first calibration level (0.1 or 0.2 ng). A check standard was analyzed nine consecutive times for intraday repeatability as well as for nine consecutive days for the interday repeatability.

Mass Load Calculation.

The mass load of NPSs was determined in four rural communities (C1, C2, C3, and C4) in Southern Illinois based on the residual level of NPSs quantified in raw wastewater samples during a typical week with no special events using the following equation

$$\text{massload} = \text{concentration}\left(\frac{\text{ng}}{L}\right) \times \text{flowrate}\left(\frac{L}{d}\right) \times \frac{1\text{mg}}{1000000\text{mg}} \times \frac{1000}{\text{population}}$$

where the mass load was expressed as mg/d/1000 people, daily average wastewater inflow was considered, and the daily population served by the WWTP was determined based on a load of ammoniacal nitrogen in raw wastewater. The ammoniacal nitrogen was measured using a portable DR 3900 spectrophotometer and Hach Company standard TNT plus method 10205 as

$$P_i = \frac{C_{NH_4-N} \times F_i}{R_{NH_4-N}} i = 1, 2, 3, \cdots\cdots \cdot, 7$$

where $C_{\mathrm{NH4–N}}$ is the concentration of ammoniacal nitrogen (mg/L), F_i is the wastewater inflow (L/d) for the ith day, and $R_{\mathrm{NH4–N}}$ is an average per-capita daily production of ammoniacal nitrogen (6900 mg/d).[6,28] The nonhuman sources of ammoniacal nitrogen could not be corrected; however, it is least affected by nonhuman sources compared to other conventional water-quality parameters including total nitrogen, total phosphorus, chemical oxygen demand, and chemical oxygen demand.[28]

RESULTS AND DISCUSSION

Figure of Merits.

The average concentration of target drugs in method blanks were below the lowest calibration level; however, methcathinone and gabapentin were measured at 2.60 and 8.10 ng/L, respectively. All reported concentrations hereby are blank-corrected. Analytical data points detected < MDL were substituted with ½ MDL values when the detection frequency is ≥70%.

The average matrix-spiking recoveries (%) of target analytes in a randomly selected wastewater sample ($n = 2$) ranged from 46.9% (NM 2201) to 164% (methoxyacetyl fentanyl). The spiking recovery of gabapentin could not be determined due to its higher background concentration (>16 folds than the spiking level) in wastewater. Two (5IT and 3′-methyl fentanyl) of the 19 target drugs that were quantified using the internal standard of other analytes resulted in elevated spiking recoveries (201 and 254%, respectively). The average percentage recoveries of target drugs from the calibration check standards that were analyzed along with the sample extracts ranged from 84.7% (5-IT) to 140% (mCPP); however, 4-methylamphetamine and 2-methyl-4′-(methylthio)-2-morpholinopropiophenone were recovered at 49.6% and 152%, respectively.

The method detection limit of NPSs determined using seven replicates of the spiked sample matrix ranged from 0.5 ng/L (MMB-CHMICA) to 6.3 ng/L (4-methylamphetamine) (Table 1). LODs ranged from 0.1 ng/L (valeryl fentanyl) to 6.6 ng/L (para-fluorobutyryl fentanyl), whereas LOQs ranged from 0.2 ng/L (valeryl fentanyl) to 22.0 ng/L (para-fluorobutyryl fentanyl) (Table 1). The interday precision expressed as a relative standard error of the measured concentrations of drugs from the repeated analysis of a check standard solution for nine consecutive days ranged from ±0.32 (4-fluoro-isobutyryl fentanyl) to ±7.31 (5IT) whereas the intraday precision for nine consecutive runs ranged from ±0.30 (furanyl fentanyl) to ±4.19 (MAB-CHMINACA) (Table 1).

Occurrence of NPSs in Wastewater.

Nine NPSs were quantified in all four communities in Southern Illinois (Table 2, Figure 3). Methcathinone was the most frequently detected NPSs (frequency of detection, df = 100%) in all target communities followed by 2-methyl-4′-(methylthio)-2-morpholinopropiophenone (a synthetic cathinone, df = 91%) and mCPP (piperazine, df = 91%). Methcathinone was reported as the most frequently detected NPS in wastewater across Australia[23] and Italy.[11] The concentration of methcathinone in this study was found ~2–4 folds higher than in Florence and ~10–30 folds higher than in Milan, Italy.[11] 4-methyl pentedrone (a cathinone) was also detected in all samples from communities C1 and C4, whereas MAB-CHMINACA (a cannabinoid) was detected in communities C1, C2, and C3 in >75% samples. Among synthetic opioids, carfentanil was detected in all samples from communities C1 and C2. Methoxyacetyl fentanyl and 5IT (an indole) were detected >90% samples from community C4. Overall, methcathinone (11.3–34.3 ng/L) and mCPP (2.3–56.4 ng/L) were more prevalent in target communities.

Estimation of the Population in WWTP Catchments.

Wastewater based epidemiologists typically report mass loading of drugs (mg/d/1000 people) to the WWTPs utilizing de jure population provided by the WWTP operators. However, de jure population does not account for the dynamicity in the population of the community.[6,28] We recently reported that the ammoniacal nitrogen—a biomarker of the population in the community—can account for the dynamicity in the population of the community and provide a near-accurate estimation of the population over the census population.[6] Baz-Lomda et al.[29] also reported that the population estimated based on ammoniacal nitrogen was found the best (mean absolute errors <10%) anthropogenic proxy to determine the population of WWTP catchment area in Norway among other potential predictors such as drinking water production and electricity consumption. The ammoniacal based population estimation of Oslo was >4% higher than the "de jure" population.[29] In this study, the near-accurate population in WWTP catchments was determined using a load of ammoniacal nitrogen in raw wastewater (Table 3).

Mass Loading of NPS.

The amount of NPS residues influx to the WWTP was determined and reported as the mass loading (mg/d/1000 people) (Figure 4). The mass load of methcathinone ranged from 2.98 ± 0.17 mg/d/1000 people in C1 to 21.1 ± 1.31 mg/d/1000 people in C3. Synthetic synthetic cathinones are among the most consumed NPSs[11] and typically consumed as the "pure" form or "adulterants" in conventional illicit drugs, such as "ecstasy" pills.[30] The actual amount of methcathinone consumed could be higher as synthetic cathinones undergo extensive metabolism in the human body[15,31] as well as in-sewer transformation including the reduction of a β-keto group, demethylenation, and demethylation.[32]

The mass load of the most prevalent cathinone (methcathinone) in community C3 was 3–7 folds higher than in C1 and C2, whereas the mass load of piperazine (mCPP) was >2 folds higher in C1 and C2 than in C3. The population is more diverse in community C3 including ~50% of college students than in community C1 and C2. mCPP was prevalent in all target communities (1.23 ± 0.32 to 15.0 ± 0.5 mg/d/1000 people) in this study. Causanilles et al.[21] reported mCPP qualitatively in wastewater during a street festival in Amsterdam, Netherland. mCPP was not detected across China; however, benzylpiperazine was prevalent at 3–10 mg/d/1000 people.[22] To our knowledge, this is the first quantitative report of mCPP in wastewater.

5IT (a stimulant, 9.75 ± 2.72 mg/d/1000 people) and 4-methyl pentedrone (a cathinone, 3.38 ± 0.18 mg/d/1000 people), and methoxyacetyl fentanyl (1.36 ± 0.28 mg/d/1000 people), were more prevalent in community C4. NPSs are also considered a cheap-substitute of conventional drugs of abuse; therefore, more prevalent in socially marginalized communities.[10] Community C4 is relatively a socially marginalized community with >30% residents below the poverty line. The mass load of MAB-CHMINACA, the only cannabinoid quantified, was up to 2.59 ± 0.56 mg/d/1000 people in community C3. To our knowledge, this is the first quantitative report of MAB-CHMINACA in wastewater. In a nationwide survey among 45 400 high school senior students, synthetic cannabinoids were the third most widely abused class of drugs in the U.S.[33] The carfentanil was the only synthetic opioid quantified in all wastewater samples in community C1 (0.51 ± 0.03 mg/d/1000 people) and C2 (0.41 ± 0.04 mg/d/1000 people). Carfentanil is ~100 folds more potent than fentanyl. The occurrence of carfentanil in this study (C1 and C2) was ~300 folds lower than the occurrence of fentanyl (152 mg/d/1000 people) in two communities in adjoined state of Kentucky.

Unlike established illicit or prescribed drugs of potential abuse, the pharmacokinetic profiles of most of the NPS's excretion are still unknown.[11,34] As a result, all quantitative WBE studies are limited to reporting the mass loading of NPSs to the WWTPs. The near-accurate estimation of the prevalence of NPSs would require to normalize mass load with the excretion rate of target NPSs (or their stable metabolites), the stability of NPS biomarkers in wastewater, and the typical does of the NPSs. To our knowledge, no wastewater stability data are available of target NPSs; however, only one study reported methcathinone's human excretion rate of 36% as a parent drug.[35] The excretion rate corrected mass load of methcathinone were 8.29 ± 0.46 mg/1000 people, 16.6 ± 2.63 mg/1000 people, 58.6 ± 3.63 mg/1000 people, and 26.1 ± 3.00 mg/1000 people at the target communities C1, C2, C3, and C4, respectively. Further study on the pharmacokinetic profiles of the NPSs execration as well as the stability of NPSs in sewer networks and the wastewater samples is inevitable to determine the prevalence of community consumption of NPSs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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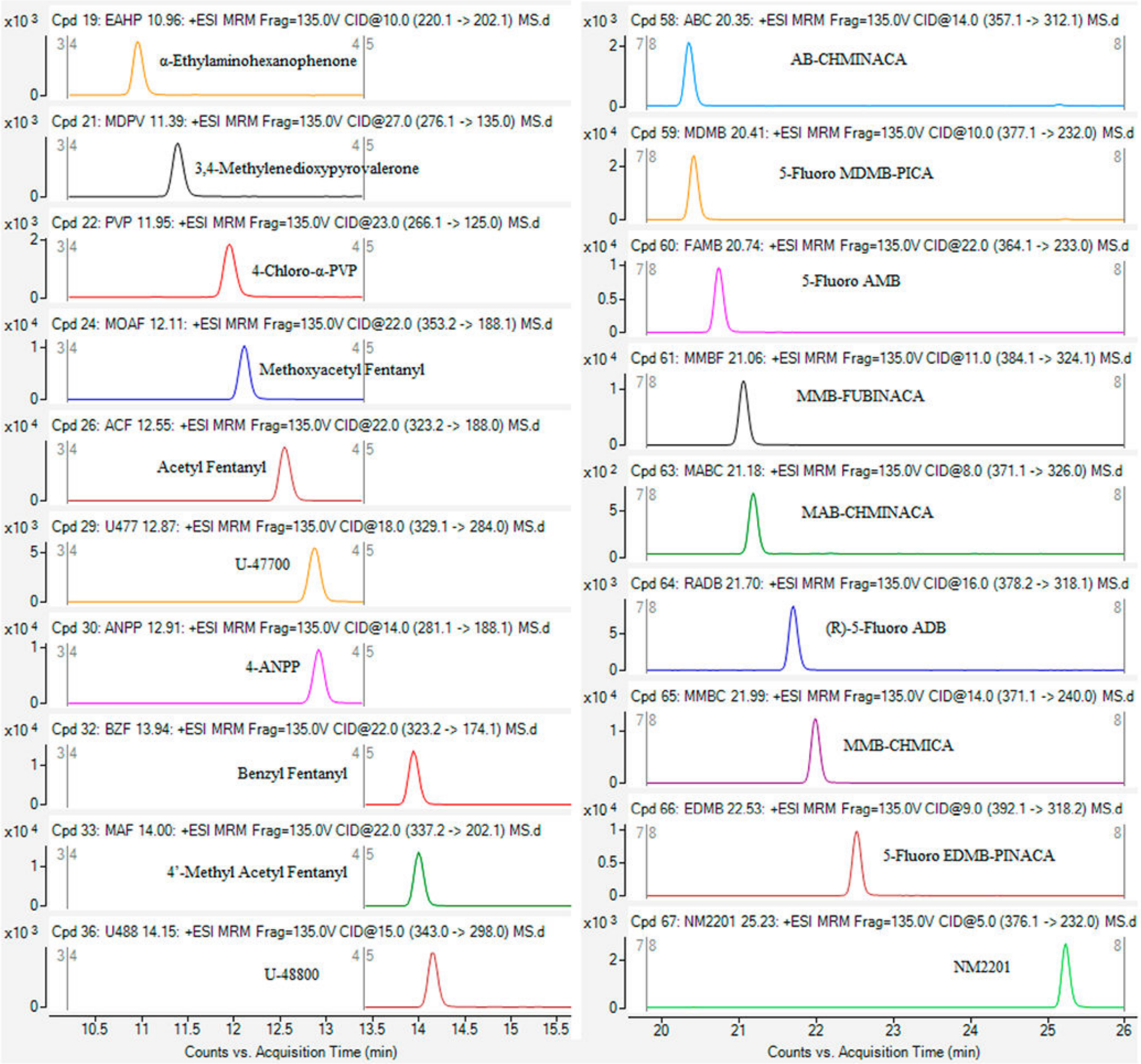

Figure 1.

Schematic diagram showing the chromatographic separation of select drugs in a drug-spiked (50 or 100 ng) wastewater extract. All other target drug's chromatograms are presented in SI Figure S1.

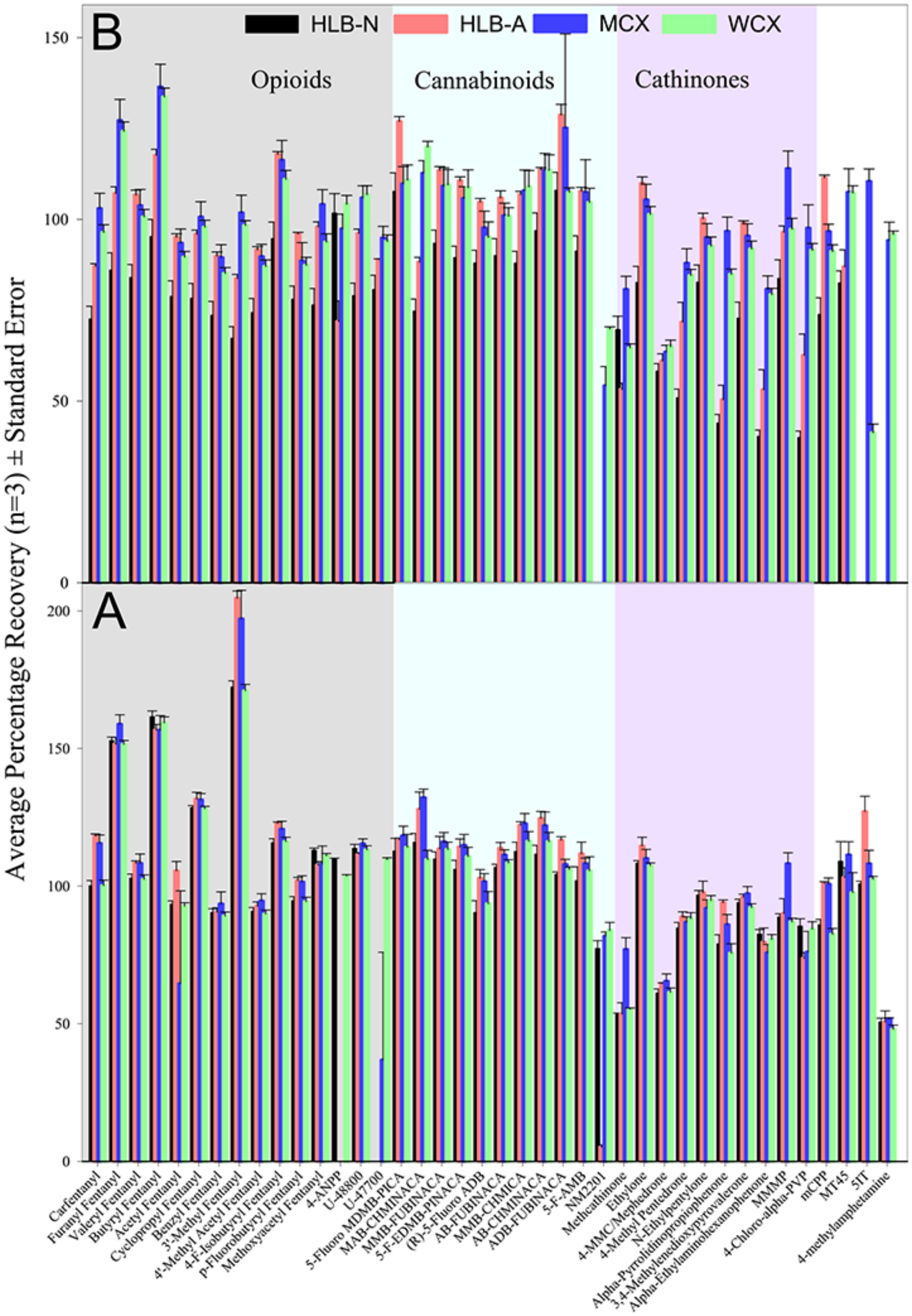

Figure 2.

Absolute spiking recoveries of target NPSs (*n* = 3) at (A) 50 or 100 ppb and (B) 2 or 4 ppb using HLB-N (pH ~ 7 sample using HLB), HLB-A (pH ~ 2 sample using HLB), MCX, and WCX cartridges. mCPP: 1-(3-chlorophenyl) piperazine; MMMP: 2-methyl-4′-(methylthio)-2-morpholinopropiophenone; 5IT: 5-(2-aminopropyl) Indole.

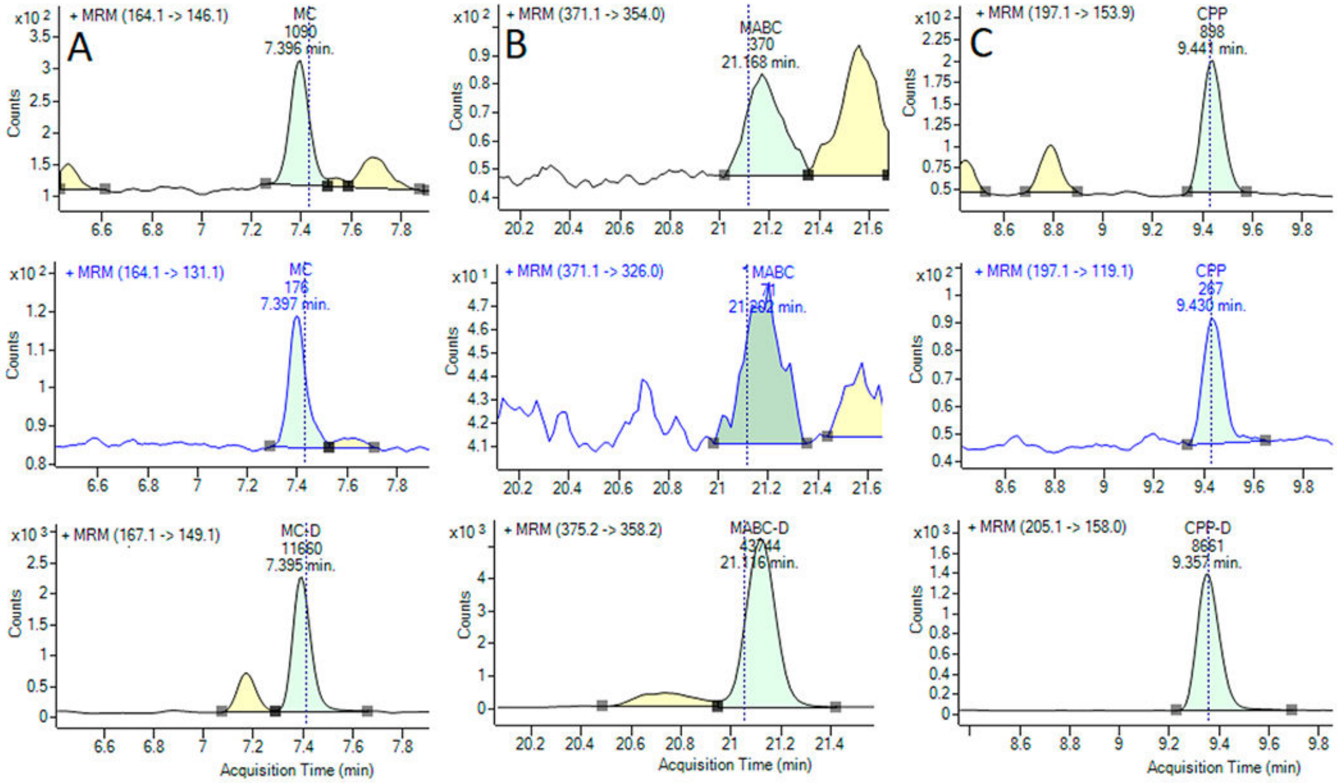

Figure 3.

Representative chromatograms of selected drugs quantified in wastewater samples. (A) methcathinone; (B) MAB-CHMINACA; (C) mCPP [1-(3-chlorophenyl) piperazine]. The top chromatogram corresponds to the quantitative MS/MS transition; middle one corresponds to the qualitative MS/MS transition; and the bottom one corresponds to the internal standard.

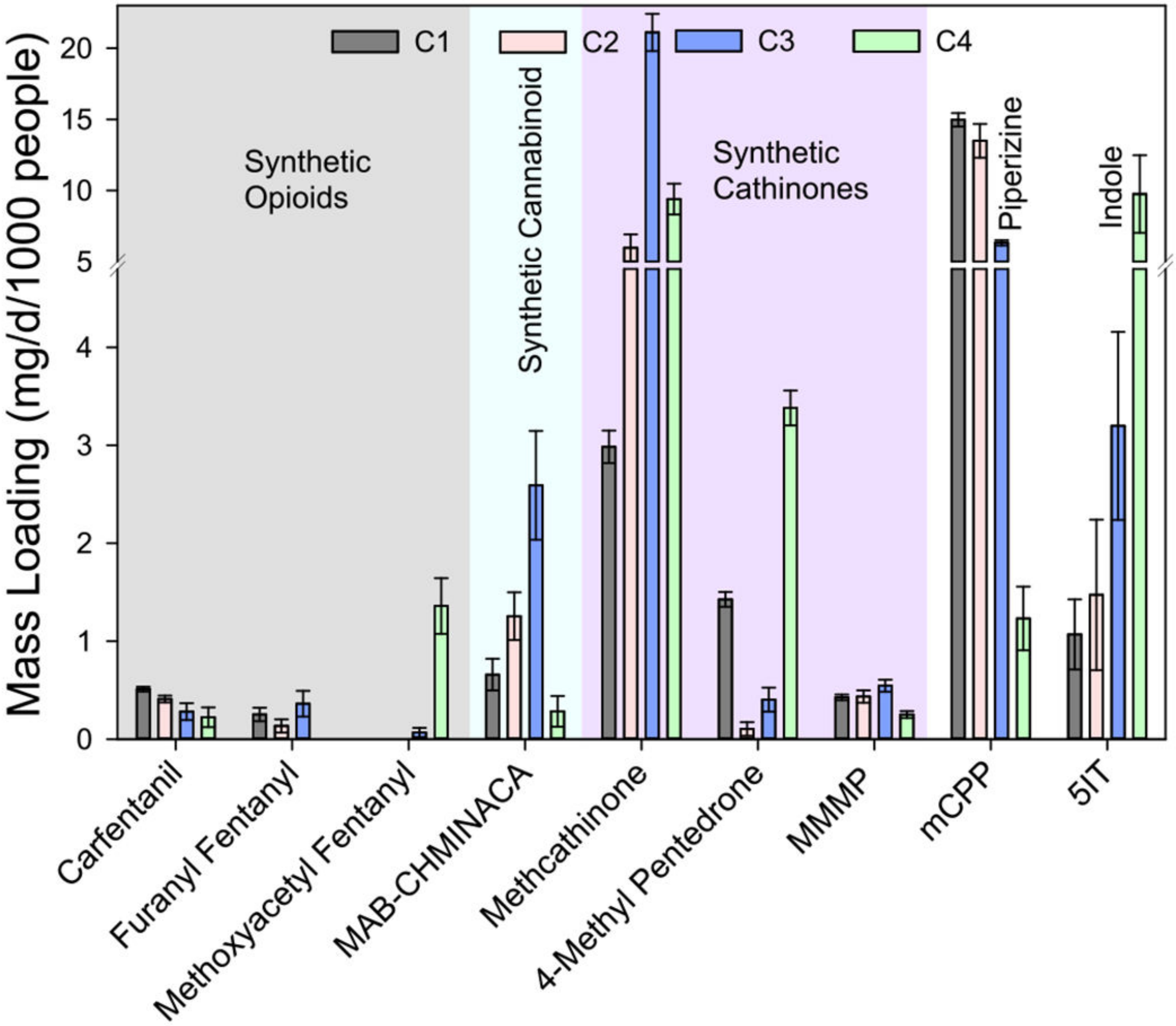

Figure 4.

Average mass loading (mg/d/1000 people) of quantified NPSs to the WWTPs in four rural communities in Illinois. MMMP: 2-methyl-4′-(methylthio)-2-morpholinopropiophenone; mCPP: 1-(3-chlorophenyl) piperazine; 5IT: 5-(2-aminopropyl) Indole.

Table 1.

Select Figure of Merits Including the Method Detection Limits (MDLs), Limits of Detection (LODs), Limits of Quantification (LOQs), and Repeatability

analytes	MDL (ng/L)	95% confidence interval (ng/L)	LOD (ng/L)	LOQ(ng/L)	intraday precision (*n* = 9)[a]	interday precision (*n* = 9)[a]
Synthetic Opioids/Analgesics carfentanil	1.00	0.70–2.30	0.50	1.50	0.71	1.16
furanyl fentanyl	1.50	1.00–3.30	0.90	3.20	0.30	0.76
valeryl fentanyl	1.00	0.70–2.20	0.10	0.20	0.50	0.58
butyryl fentanyl	1.10	0.70–2.50	0.30	0.90	0.68	0.85
acetyl fentanyl	0.70	0.50–1.60	0.10	0.40	0.36	0.92
cyclopropyl fentanyl	0.90	0.60–2.00	0.20	0.70	0.60	0.70
benzyl fentanyl	0.90	0.60–2.00	0.10	0.50	0.65	1.10
3′-methyl fentanyl	1.30	0.80–2.90	0.10	0.30	0.53	0.48
4′-methyl acetyl fentanyl	0.80	0.50–1.70	0.10	0.20	0.73	0.67
4-fluoro-isobutyryl fentanyl	1.30	0.80–2.80	0.10	0.0.20	2.19	0.32
para-fluorobutyryl fentanyl	0.70	0.50–1.60	6.60	22.0	1.14	0.61
methoxyacetyl fentanyl	0.70	0.40–1.50	0.10	0.20	0.58	0.73
4-ANPP	1.30	0.90–3.00	0.40	1.20	0.66	1.22
U-48800	1.20	0.80–2.60	0.10	0.30	0.69	1.10
U-47700	1.00	0.60–2.20	0.30	1.00	0.44	0.67
Synthetic Cannabinoids						
5-fluoro MDMB-PICA	1.00	0.60–2.20	0.20	0.70	0.94	2.57
MAB-CHMINACA	1.80	1.10–3.90	6.30	21.0	4.19	3.29
MMB-FUBINACA	2.90	1.90–6.40	0.80	2.60	1.56	2.33
5-fluoro EDMB-PINACA	2.30	1.50–5.10	0.10	0.40	1.98	2.93
(R)-5-fluoro ADB	1.10	0.70–2.40	0.40	1.20	1.60	1.09
AB-FUBINACA	0.90	0.60–1.90	2.20	7.30	2.09	1.62
MMB-CHMICA	0.50	0.30–1.10	0.20	0.60	0.66	1.47
AB-CHMINACA	1.70	1.10–3.80	1.30	4.20	2.04	2.02
ADB-FUBINACA	0.90	0.50–1.90	1.0.	3.50	3.32	1.41
5-fluoro AMB	0.80	0.50–1.70	0.20	0.50	1.05	1.38
NM2201	2.80	1.80–6.20	0.40	1.30	1.37	2.98
Synthetic Cathinones						

analytes	MDL (ng/L)	95% confidence interval (ng/L)	LOD (ng/L)	LOQ(ng/L)	intraday precision (*n* = 9)[a]	interday precision (*n* = 9)[a]
methcathinone	1.20	0.80–2.70	0.70	2.20	0.97	0.99
ethylone	1.0.	0.70–2.30	0.30	1.00	0.33	0.67
4-MMC (mephedrone)	1.60	1.10–3.60	1.00	3.30	0.78	3.15
4-methyl pentedrone	1.60	1.00–3.60	2.40	7.80	1.73	2.04
N-ethylpentylone	1.70	1.10–3.70	2.30	7.70	0.38	0.81
α-pyrrolidinopropiophenone	1.50	1.00–3.30	0.50	1.50	0.40	1.40
3,4-methylenedioxypyrovalerone	0.90	0.60–2.10	0.40	1.40	0.45	0.76
α-ethylaminohexanophenone	1.40	0.90–3.20	2.50	8.40	0.61	2.53
2-methyl-4′-(methylthio)-2-morpholinopropiophenone	0.60	0.40–1.30	0.20	0.80	0.40	1.52
4-chloro-*α*-PVP	1.30	0.90–2.90	0.40	1.30	0.56	1.27
Piperazines						
1-(3-chlorophenyl) piperazine (mCPP)	2.20	1.40–4.80	0.90	2.90	1.93	1.80
1-cyclohexyl-4-(1,2-diphenylethyl) piperazine (MT-45)	0.80	0.50–1.90	0.10	0.30	0.59	0.65
Indole						
5-(2-aminopropyl) Indole (5IT)	3.30	2.10–7.20	3.30	10.9	1.32	7.31
Amphetamine						
4-methylamphetamine	6.30	4.10–13.9	1.70	5.50	0.67	3.38
Anticonvulsants						
clonazepam	3.40	2.20–7.50	1.90	6.30	0.78	1.61
gabapentin	248	159–546	3.40	11.2	1.64	2.45

[a] Relative standard errors (±).

Table 2.

Average Concentration of Quantified NPSs in Raw Wastewater from Four Communities in Southern Illinois[a]

analytes	C1 (ng/L ± SE)	C2 (ng/L ± SE)	C3 (ng/L ± SE)	C4 (ng/L ± SE)
Synthetic Opioids carfentanil	1.9 ± 0.1	1.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.2
furanyl fentanyl	1.0 ± 0.3	0.6 ± 0.3	0.6 ± 0.2	ND
methoxyacetyl fentanyl	<MDL	<MDL	±0.1	2.3 ± 0.5
Synthetic Cannabinoids MAB-CHMINACA	2.5 ± 0.6	4.0 ± 0.9	4.4 ± 1.0	0.6 ± 0.3
Synthetic Cathinones methcathinone	11.3 ± 0.8	17.8 ± 1.3	34.3 ± 2.1	16.9 ± 2.6
4-methyl pentedrone	5.3 ± 0.3	0.4 ± 0.3	0.7 ± 0.2	5.8 ± 0.2
MMMP	1.6 ± 0.1	1.3 ± 0.1	0.9 ± 0.1	0.4 ± 0.05
Piperazine mCPP	56.4 ± 2.7	42.1 ± 2.6	10.3 ± 0.3	2.3 ± 0.6
indole 5IT	4.3 ± 1.5	3.6 ± 1.4	5.3 ± 1.6	19.2 ± 6.3
Anticonvulsant gabapentin	131,000 ± 7600	58,600 ± 5100	25,500 ± 1150	12,100 ± 1370

[a] MDL: method detection limit; ND: non detected, MMMP: 2-methyl-4′-(methylthio)-2-morpholinopropiophenone; mCPP: 1-(3-chlorophenyl) piperazine; 5IT: 5-(2-aminopropyl) indole.

Table 3.

Estimated Population of Four Communities in Southern Illinois Based on a Load of Ammoniacal Nitrogen in Raw Wastewater

communities	census estimation[a]	NH_4–N (mg/L)[b]	NH_4-N-based population[b]
C1	7882	23.2 ± 1.9	9283 ± 397
C2	17 620	21.1 ± 2.1	19,597 ± 799
C3	20 000[c]	10.3 ± 0.5	16,253 ± 976
C4	2188	10.8 ± 0.9	2980 ± 239

[a] Census annual estimate of the resident population by the U.S. Census Bureau in 2018.

[b] Average ± standard error.

[c] WWTP operator's estimation.