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Article

First Detection of Xylazine in Texas Wastewater and Its Association with Fentanyl Use

Katherine M. Joseph, Xuan Qin, Dhvani Parikh, Feng Li, John Balliew, Kristina D. Mena, and Fuqing Wu*

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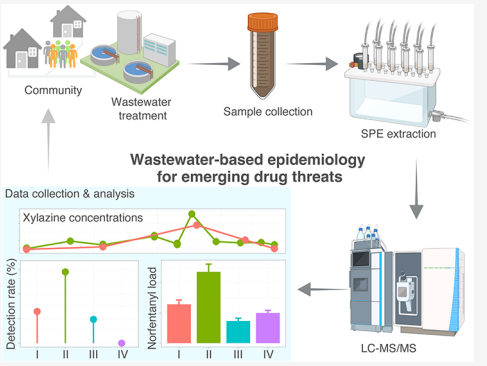
ABSTRACT: The United States is dealing with an escalating drug overdose crisis intensified by emerging threats, particularly xylazine, a veterinary sedative increasingly found as an adulterant in illicit drug supplies. To investigate the prevalence of xylazine and examine its spatial correlation with fentanyl consumption, we employed wastewater-based epidemiology to analyze 124 samples from four wastewater treatment plants serving El Paso, Texas, over 13 months (June 2023 to July 2024). Samples were analyzed for xylazine and norfentanyl (fentanyl metabolite) using liquid chromatography−tandem mass spectrometry, with population-normalized mass loads calculated for spatial comparisons. Xylazine was detected in three of four treatment plants, showing detection rates of 29.0, 12.9, 9.7, and 0% across different sewersheds. All samples tested positive for norfentanyl, confirming city-wide fentanyl use. Peak population-normalized loads reached 110 ± 40 μg/day/1000 people for xylazine and 2400 ± 300 μg/day/1000 people for norfentanyl. Statistical analysis revealed that sewersheds with higher xylazine detection rates exhibited significantly elevated norfentanyl loads ($p < 0.05$), suggesting a community-level association between xylazine and fentanyl use. This study provides the first evidence of xylazine in Texas wastewater and reveals spatially clustered distribution patterns that may inform targeted public health interventions to mitigate the growing threat of xylazine within the opioid crisis.



KEYWORDS: *xylazine, fentanyl, illicit drugs, wastewater-based epidemiology, border communities*

1. INTRODUCTION

Xylazine is a veterinary sedative and an emerging threat in the drug overdose crisis.[1,2] Its presence in overdose deaths has grown steadily since its first identification as an adulterant in Puerto Rico in the early 2000s.[3] Xylazine (known as "tranq" in street drugs) is often found mixed with fentanyl and other drugs.[4−7] The addition of xylazine extends the effects of opioids, while also increasing the risk of fatal overdose.[8,9] Xylazine is not an opioid. The treatment of Narcan/Naloxone against fentanyl is ineffective against xylazine.[10,11] Recognizing this threat, the White House Office of National Drug Control Policy (ONDCP) declared xylazine as an emerging threat in April 2023.[12]

There are many challenges in addressing the xylazine threat. First, there are limited data on its prevalence and distribution, as xylazine is not routinely included in standard toxicology screens.[13−15] This leads to under-reporting and an incomplete understanding of its true impact. Second, traditional surveillance methods, such as surveys and clinical reports, often suffer from delays of months or even years,[16−18] which hinders rapid response. Third, the illicit use of xylazine makes it difficult to accurately assess consumption patterns through conventional approaches.[19] Finally, the varying legal status of xylazine across jurisdictions complicates coordinated monitoring and intervention efforts. Currently, only Florida has explicitly banned xylazine, while it remains unregulated in many other states.[20−22] These challenges impede timely and effective public health responses to address the xylazine threat within the broader opioid crisis.

Wastewater-based epidemiology (WBE) offers a promising approach to overcoming many challenges in tracking drug use at the community level.[23,24] Innovative methods have analyzed wastewater to detect and quantify drugs and their metabolites, providing near real-time data on drug use trends.[25,26] Utilizing excretion rates and wastewater flow data, wastewater data can be used to estimate drug consumption levels in the community or "sewershed." The noninvasive nature of wastewater sampling also allows for continuous monitoring without the delays associated with traditional surveillance methods.[27] Researchers have successfully applied WBE to monitor the use of opioids, stimulants, psychoactive medications, and other

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drugs.[28−36] For example, a two-year wastewater surveillance program in rural Massachusetts consistently detected ten opioids and stimulants and identified increases in drug use during the COVID-19 pandemic, which highlights the value of longitudinal wastewater testing for evolving drug use patterns.[26] More recently, a study in Kentucky detected xylazine in wastewater samples across the state.[25] These studies demonstrate the potential of the WBE in providing timely insights into emerging drug trends and complementing existing health monitoring programs.

Our study focuses on El Paso, Texas, the sixth largest city in the state, and a US−Mexico border city known for its major trade hub. El Paso County's overdose death rate nearly doubled from 11.3 to 21.8 per 100,000 residents between 2018 and 2019 and 2022−2023, with 58% of these deaths involving fentanyl.[37] The prevalence of xylazine use in El Paso is unclear, but the city is located within a region of concern based on Drug Enforcement Administration (DEA) seizure reports indicating that xylazine is increasingly found mixed into fentanyl.[38,39] Furthermore, a DEA Joint Intelligence Report showed a 1127% increase in deaths involving xylazine across the southern United States from the year 2020 to 2021.[21] Given these concerning trends, this study aimed to detect the presence of xylazine and examine its spatial association with fentanyl use in El Paso's wastewater over 14 months (June 2023 to July 2024). This investigation is critical for understanding the evolving drug trends in this important border community and is the first wastewater-based xylazine surveillance study in Texas.

2. MATERIALS AND METHODS

2.1. Wastewater Sample Collection

The raw influent wastewater samples were collected from the four wastewater treatment plants (WWTPs) in El Paso, Texas, from June 26, 2023, to July 15, 2024. The four WWTPs serve ∼751,982 total customers in the city.[40,41] The WWTPs were anonymized for this paper and were identified as WWTPs I, II, III, and IV. Composite samples were collected using HACH AS950 All-Weather Refrigerated Samplers programmed to collect aliquots every 30 min over a 24-h period. Aliquots of 500 mL were shipped overnight on ice to the laboratory and stored at −20 °C until analysis. Sample collection and shipment occurred consistently on Mondays throughout the study period based on agreement with the facility. A total of 124 samples were collected and analyzed in this study (31 from each WWTP, Table S1). Sampling frequency was monthly during 2023 (36 samples) and increased to weekly during 2024 (88 samples) due to improved resource availability.

2.2. Sample Preparation and Quantification with LC-MS/MS

Raw wastewater samples (35 mL) were centrifuged, transferred, and further filtered with a 0.22 μm poly(ether sulfone) membrane (Millipore Sigma, #SCGP00525) to remove remaining detritus. The filtrate (30 mL) was spiked with 100 ng/L isotopically marked standards (xylazine-d6 and fentanyl-d5, Cayman Chemical, #38353 and #14721) and extracted using solid-phase extraction (SPE) cartridges on an extraction manifold (Waters, #WAT200606). Specifically, samples were acidified to pH 2.5 using hydrochloric acid (HCl, Millipore Sigma, #320331) and then loaded onto an Oasis MCX cartridge (Waters, #186000255), which was preconditioned sequentially with HPLC-grade methanol (Millipore Sigma, #A456), HPLC-grade water (Millipore Sigma, #W64), and 0.1% HCl in HPLC water (v/v). A vacuum pump was applied. The sample bottles were sequentially rinsed with water and 0.1% (v/v) HCl, and the rinse solutions were also loaded onto the cartridge.

Methanol was used to wash the cartridges, and the vacuum was applied for 30 s to eliminate any residual wash solvent. The cartridge was then air-dried at room temperature. The analytes were eluted from the cartridge using a 2 mL mixture of MTBE (*tert*-butyl methyl ether)/isopropanol/ammonium hydroxide at a ratio of 78:20:2 (v/v/v, Fisher Scientific, #E127). The vacuum was applied for 30 s to flush out any residual elution buffer. The eluent was collected in 15 mL tubes, dried with a gentle nitrogen gas flow, and reconstituted with 150 μL of 50% aqueous methanol, which was further vortexed and centrifuged at 15,000 rcf for 15 min. This SPE process resulted in a 200-fold concentration (from 30 mL to 150 μL).

The supernatants were transferred to sample vials, and 5 μL was injected into a Thermo Vanquish UHPLC (Thermo Fisher Scientific) coupled with a Thermo TSQ Quantis tandem mass spectrometer (MS/MS) for analysis and quantification. The analytes and the internal standards (IS) were separated on an Agilent XDB-C18 column (4.6 mm × 50 mm, 3 μm) and eluted by a water−acetonitrile mobile phase system (both containing 0.1% formic acid, v/v) with a gradient from 15 to 95% organic phase within a 6 min run. The flow rate was set at 0.3 mL/min, and the column temperature was set at 40 °C. Analytes and IS were monitored in the selected reaction monitoring (SRM) mode coupled with a positive electrospray ionization (ESI) source. The ion spray voltage was set at 3500 V. Ultrahigh purity nitrogen was used as the sheath gas (50 arbitrary units), auxiliary gas (10 arbitrary units), and sweep gas (1 arbitrary unit), and ultrahigh purity argon was used as the collision gas. The temperatures of the ion transfer tube and vaporizer were 325 and 350 °C, respectively. Signals from 1.5 to 5.5 min were recorded by the mass spectrometer. The retention times of the analytes and IS, along with quantification SRM ion transitions, are listed in Table S2. The LC-MS/MS instrument parameters are also listed in Table S3.

Calibration standards at six concentration levels (0.2−10 ng/L for xylazine and 1−50 ng/L for norfentanyl) were prepared for each analytical batch by spiking HPLC-grade water with certified reference standards of xylazine and norfentanyl. These concentrations are the analyte levels in water before SPE, and the ranges were selected based on concentration ranges measured in our wastewater samples. HPLC-grade water blanks served as negative controls. Method validation included assessment of linearity, intraday and interday precision, and accuracy and recovery. Limits of detection (LOD) and lower limit of quantification (LLOQ) were determined using signal-to-noise ratios of 3:1 and 10:1,[42] respectively. The LOD and LLOQ are the raw concentrations spiked into water before SPE. Quality control (QC) samples at low, medium, and high concentrations were analyzed in triplicate within each batch to monitor the method performance and ensure data quality throughout the study period. The SPE recoveries were calculated as follows: (1) peak area 1 (SPE-ed): Xylazine and norfentanyl of 3 QC levels were spiked into water and processed via SPE; (2) peak area 2 (Post SPE): Drug-free water was processed via SPE, and the samples were reconstituted with 50% methanol/water containing corresponding concentrations of xylazine and norfentanyl (adjusted for the 200-fold concentration factor). Recovery = peak area 1/peak area 2*100%. Isotopically marked IS were added together with the analytes, and the recoveries were calculated in the same method.

2.3. Calculation of Xylazine and Norfentanyl Mass Loads and Per-Capita Consumption

The xylazine and norfentanyl mass loads were calculated by multiplying the measured concentrations (ng/L) by the daily influent flow volume (eq 1).[24,32,43] Daily wastewater flow data (million gallons per day, MGD) were provided by the wastewater treatment plants. One MGD equals 3,785,412 L. The per-capita consumption (per 1000 people) was then determined by dividing the mass load by the population size in each sewershed (eq 2). This estimation does not account for the drug excretion rate into urine and stool, which is approximately 70% for xylazine, based on studies conducted in rats,[44] and 91% for norfentanyl in humans.[43,45] To date, no corresponding human excretion data for xylazine have been reported. All of the per-capita consumption values are reported with significant figures to account for the uncertainties associated with sampling.[46−48]

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Table 1. Method Validation Parameters and Extraction Recovery Rates

targets	LOD (ng/L)	LLOQ (ng/L)	linear range (ng/L)	nominal concentration (ng/L)	peak area		Recovery (%)
					SPE-ed	post SPE	
				0.5	616	955	64.52
Xylazine	0.1	0.2	0.2−10	2	2910	6139	47.40
				8	10,491	25,303	41.46
Norfentanyl				2	1687	5765	29.26
	0.5	1	1−50	10	7741	29,866	25.92
				40	27,865	102,534	27.18
Fentanyl-d5	\	\	\	100	779,952	1,319,733	59.10
Xylazine-d6	\	\	\	100	158,873	363,026	43.76

Table 2. Intraday Precision and Accuracy

	nominal concentration (ng/L)	test 1 (ng/L)	test 2 (ng/L)	test 3 (ng/L)	average (ng/L)	precision (%)	accuracy (%)
	0.5	0.52	0.43	0.50	0.48	9.32	−3.37
Xylazine	2	2.09	2.08	1.98	2.05	2.92	2.58
	8	7.31	8.31	7.95	7.86	6.43	−1.81
Norfentanyl	2	2.10	1.87	2.24	2.07	8.88	3.51
	10	8.77	9.70	9.50	9.33	5.24	−6.75
	40	39.97	41.53	41.83	41.11	2.43	2.78

Table 3. Interday Precision and Accuracy

	nominal concentration (ng/L)	test 1 (ng/L)	test 2 (ng/L)	test 3 (ng/L)	average (ng/L)	precision (%)	accuracy (%)
	0.5	0.52	0.53	0.51	0.52	1.87	3.93
Xylazine	2	2.09	1.88	2.16	2.04	7.14	2.02
	8	7.31	7.16	6.92	7.13	2.78	−10.90
Norfentanyl	2	2.10	2.01	2.24	2.12	5.36	5.79
	10	8.77	8.59	8.92	8.76	1.88	−12.39
	40	39.97	43.43	44.92	42.77	5.93	6.93

$$\text{mass load of drug}(\mu\text{g/day}) = (\text{measured concentration}(\mu\text{g/L}) \times \text{daily flow (liters per day)}) \quad (1)$$

$$\text{drug per capita consumption}(\mu\text{g/day/1000 people}) = \frac{\text{mass load of drug}}{\text{population size}} \times 1000 \quad (2)$$

2.4. Contextual Data Collection about Mortality-Involving Xylazine and Local Veterinary Clinics

Xylazine-involved mortality data were obtained from the National Forensic Laboratory Information System (NFLIS).[49] The NFLIS data were queried to investigate reported xylazine-involved mortalities from 2019 to 2023 from the NFLIS Public Data Query System (DQS). The search term "xylazine" was applied for those years, and the numbers of related drug reports were collected at the state (Texas) and national levels for analysis.

To assess the potential relationship between wastewater detection patterns and veterinary xylazine use, we mapped the locations of registered veterinary clinics in El Paso to their corresponding sewersheds. Specifically, the addresses and service details of all veterinary clinics in the city of El Paso were obtained from the El Paso Veterinary Medical Association (EPVMA) Web site. These addresses were geocoded to a map with their longitudinal and latitudinal coordinates by using Google Maps. Each clinic was then matched to its respective sewersheds based on its geographic location using the software ArcGIS Pro (version 3.6). To assess the potential spatial impact of hospitals on the norfentanyl load in the wastewater, we mapped the locations of hospitals in El Paso to their corresponding sewersheds. This information was obtained through public records and geocoded through ArcGIS Pro in the same manner as for veterinary clinics.

2.5. Statistical Analysis

The Welch two-sample t test was employed to assess differences in norfentanyl per-capita consumption loads between sewersheds. This test was chosen for its robustness in comparing groups with potentially unequal variances. A significance level of $\alpha = 0.05$ was used, with p-values below this threshold indicating statistically significant differences between compared sewersheds. All statistical analyses were performed using R software (version 4.3.1), with the "t.test()" function for the Welch t test.

3. RESULTS

3.1. Method Validation and Analytical Performance

To validate the analytical method, we validated the parameters, including LOD, LLOQ, and linearity, intra- and interday accuracy and precision, and recoveries for both target analytes according to the guidelines of FDA[42] (Tables 1−3). Xylazine and norfentanyl peaks were not detected in the blank matrix (HPLC water), showing good specificity of the method (Figures S1 and S2). LODs were determined as 0.1 ng/L for xylazine and 0.5 ng/L for norfentanyl, with corresponding LLOQs of 0.2 ng/L and 1 ng/L (all were the concentrations spiked into HPLC water before SPE), respectively. Linear calibration ranges were established at 0.2−10 ng/L for xylazine and 1−50 ng/L for norfentanyl according to their concentrations in the wastewater samples with good linearities ($R^2 > 0.99$) (Figure S3). Low, medium, and high levels of QC (0.5, 2, and 8 ng/L for xylazine; 2, 10, and 40 ng/L for norfentanyl) were chosen across the calibration curve ranges and were used

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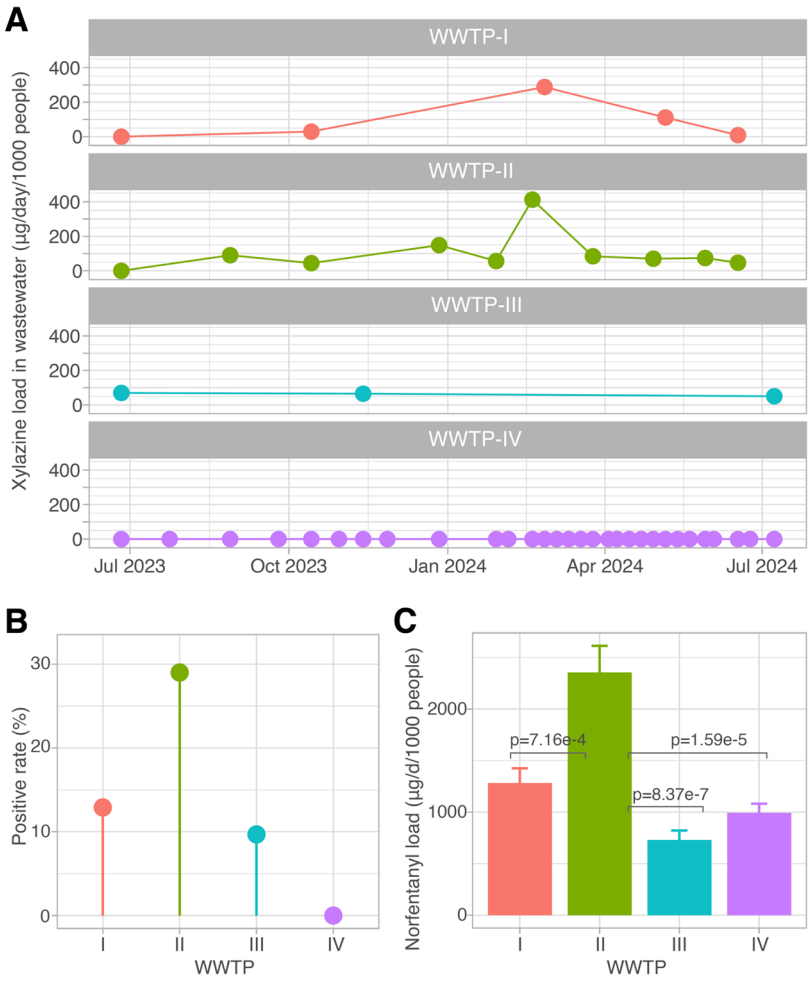

Figure 1. Xylazine detection in wastewater and its association with fentanyl use. (A) Temporal trends of xylazine load per capita across the four sewersheds in El Paso, Texas (points below the limit of detection for samples from WWTP-I, II, and III were omitted for visualization). (B) Positive detection rates of xylazine in wastewater from each sewershed. (C) Mean norfentanyl per-capita load across sewersheds. Xylazine and norfentanyl loads were calculated by multiplying the measured concentrations by the wastewater flow volume and normalized by the population size. Error bars represent standard error (n = 31 samples for each WWTP). P-value was obtained via the Welch two-sample t test.

in the following method validation processes (Tables 2 and 3). We also measured the recovery of the solid-phase extraction process, which varied across concentration levels, ranging from 41.46 to 64.52% for xylazine and from 25.92 to 29.26% for norfentanyl. The average recoveries of xylazine-d6 and fentanyl-d5 were 43.76 and 59.10% (Table 1), respectively, similar to the corresponding analytes.

We also evaluated the intraday precision and accuracy using 3 QC levels prepared on the same day (Table 2), and interday precision and accuracy using 3 QC levels over three independent analytical runs on different days (Table 3). Within the same day, the precision (coefficient of variation) ranged from 2.92 to 9.32% for xylazine and from 2.43 to 8.88% for norfentanyl; accuracy (deviation from nominal QC concentrations) ranged from −1.81 to 2.58% for xylazine and from −6.75 to 3.51% for norfentanyl. The interday precision ranged from 1.87 to 7.14% for xylazine and 1.88 to 5.93% for norfentanyl; the interday accuracy ranged from 3.93 to −10.90% for xylazine and 6.93 to −12.39% for norfentanyl. Overall, the method demonstrated sufficient precision (within 15% for all levels) and accuracy (within ±15% for all levels) for reliable quantification of xylazine and norfentanyl in wastewater samples.[42,50]

3.2. Xylazine Detection and Heterogeneous Prevalence across Sewersheds

With the established method, we analyzed a total of 124 wastewater samples for xylazine from four wastewater treatment plants serving the entire border city of El Paso, Texas, over a 13-month period from June 2023 to July 2024. To account for the varying flow rates and population sizes across sewersheds, xylazine data are presented as population-normalized mass loads (μg/day/1000 people) rather than raw concentrations. LC-MS/MS results revealed xylazine detection in three of the four WWTPs, with spatial heterogeneity in the detection frequency and load across sewersheds (Figure 1A).

Specifically, detection rates varied substantially between sites: WWTP-II showed the highest detection frequency with 9 positive samples out of 31 (29.03%), followed by WWTP-I with 4 positive detections (12.90%), and WWTP-III with 3 positive detections (9.70%, Figure 1B). WWTP-IV had no detectable xylazine throughout the entire monitoring period (0%). Among the positive detections, WWTP-II had the highest xylazine loads (110 ± 40 μg/day/1000 people), with a peak of 410 μg/day/1000 people on February 19, 2024. WWTP-I reached its maximum load of 290 μg/day/1000

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people on February 26, 2024. The sporadic positive detections at WWTP-III remained at relatively low levels (50−70 μg/day/1000 people). These results demonstrate positive detection of xylazine in wastewater and reveal spatial heterogeneity in usage patterns across different areas of El Paso.

3.3. Xylazine Prevalence is Associated with Fentanyl Consumption Rate

Given that xylazine is frequently found coformulated with fentanyl in street drug supplies,[4−7] we investigated potential spatial correlations between xylazine detection and fentanyl consumption across El Paso's sewersheds. We analyzed norfentanyl (a stable fentanyl metabolite) in all 124 samples to assess the fentanyl consumption patterns across the four WWTPs. Norfentanyl was detected in all samples, with loads ranging from 160 to 4700 μg/day/1000 people. This ubiquitous presence of norfentanyl suggests a high prevalence of fentanyl use throughout El Paso.

A comparison of average norfentanyl loads across sewersheds revealed a spatial pattern that closely paralleled xylazine detection frequencies (Figure 1C). WWTP-II had the highest fentanyl consumption burden with norfentanyl loads at 2400 ± 300 μg/day/1000 people, followed by WWTP-I (1300 ± 140 μg/day/1000 people), WWTP-IV (990 ± 90 μg/day/1000 people), and WWTP-III (730 ± 90 μg/day/1000 people). Statistically significant differences were found between WWTP-II and the other three WWTPs. Notably, this spatial distribution of fentanyl consumption corresponds with xylazine detection patterns, where WWTP-II showed the highest xylazine detection rates (29.0%) and loads, while WWTP-III and WWTP-IV exhibited minimal or no xylazine detection. These findings demonstrate a spatial correlation between xylazine prevalence and fentanyl consumption and suggest codistribution of these substances within El Paso's illicit drug market.

4. DISCUSSION

This study provides the first evidence of xylazine presence in wastewater samples from El Paso, Texas, a major city on the U.S.−Mexico border. We detected xylazine in three out of four wastewater treatment plants serving the city, with the highest prevalence in the WWTP-II sewershed. Notably, we observed an association between xylazine prevalence and higher fentanyl consumption rates in the same areas, suggesting a potential link between the use of these substances. These findings provide a population-level perspective on the emerging xylazine threat in a region already grappling with the opioid crisis.

The detection of xylazine in El Paso wastewater aligns with the rising trend of xylazine-involved mortality reported across the United States, particularly in the South. According to the DEA, the Southern U.S. had the highest increase in xylazine identifications and xylazine-involving overdose mortalities nationwide.[21] This is consistent with the national trend of xylazine-involved mortalities based on data from the NFLIS (Figure 2). In Texas, while xylazine-related deaths decreased starting in 2021, the total mortality in 2023 was approximately 9-fold higher than in 2019.[49] Although public records have not yet indicated xylazine-related mortalities in El Paso, the DEA has reported seizing fentanyl and xylazine mixtures within the county.[38] Our results provide direct evidence of xylazine's presence in the city's wastewater, potentially indicating a more widespread issue than previously recognized. The spatial

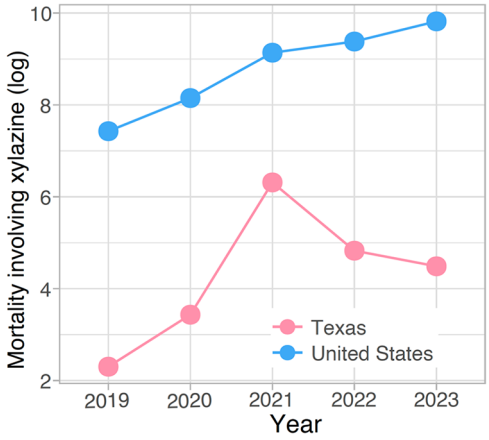

Figure 2. Xylazine-related mortality trends and study sewershed mapping with veterinary clinics. Xylazine-involved mortality trends in Texas and the United States from 2019 to 2023. Data were obtained from the NFLIS data query system (*y*-axis: natural logarithm).

heterogeneity observed in xylazine detection across sewersheds might reflect neighborhood-level drug market dynamics and socioeconomic factors.[51,52] The co-occurrence of higher fentanyl consumption levels in areas with more frequent xylazine detection is particularly concerning because the combination of these drugs is known to drastically increase the risk of fatal overdoses,[53,54] and the standard opioid antagonists like naloxone are ineffective against its sedative effects.[4,55]

This study has four limitations that should be considered when interpreting the results. First, in estimating xylazine consumption rates, we did not account for the excretion rate (approximately 70% in rats, and is unknown in humans), and potential degradation/losses in the sewer system.[44,56−58] Including these factors would likely increase our consumption rate estimates and allow for more accurate dose estimations per sewershed. Further investigation of the potential transformation pattern of xylazine in the sewer would also improve the reliability of consumption rate estimations.[59,60]

Second, the detection of xylazine and norfentanyl in wastewater may originate from multiple sources. Xylazine is used as a veterinary sedative, primarily for large animals,[61] and fentanyl has medical applications in surgery, pain management, and childbirth.[62] To contextualize our findings, we mapped all registered veterinary clinics and hospitals in the city (Table 4).[63] The majority of veterinary clinics, including those serving large animals, are located in WWTP-III and IV, and most hospitals are in WWTP-II and IV. Notably, no xylazine was detected in sewershed IV, and only 9.7% detection was observed in sewershed III (Figure 1), despite these sewersheds having the most veterinary clinics. The spatial association between xylazine and norfentanyl detection in wastewater does

Table 4. Hospitals and Registered Veterinary Clinics in El Paso, Texas

WWTP	# of hospitals	# of veterinarian clinics	# of veterinarian clinics serving large animals	# of veterinarian clinics serving small animals
I	3	4	0	4
II	8	5	1	5
III	1	14	3	13
IV	5	17	4	17

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not correspond directly to the distribution of these facilities, suggesting the observed association likely indicates human use patterns rather than facility-based sources alone. Further data collection on local veterinary xylazine use and xylazine-involved human cases would enable a more precise assessment of source contributions.

Third, xylazine metabolites were not measured in this study. A recent study from Missouri demonstrated the detection of five xylazine metabolites in human samples via LC-MS/MS,[64] which indicates that inclusion of metabolites in future wastewater analyses could provide additional resolution for source attribution and strengthen the interpretation of community-level exposure. Finally, wastewater data in this study may not fully capture localized use patterns due to population movement within the city and the daily influx of visitors. El Paso receives an average of 15,532 pedestrians, 35,712 passenger vehicles, and 2,937 commercial vehicles daily through its ports of entry.[65] Furthermore, all samples were collected on Mondays, which enhances spatial comparisons across sewersheds but may not reflect the day-of-week variability in substance use. Future studies employing stratified random sampling across multiple days[66,67] and integrating facility-level dispensing records and clinical surveillance data would be crucial for a more comprehensive assessment of temporal patterns and source contributions.

5. CONCLUSIONS

Despite these limitations, our study provides the first detection of xylazine in Texas wastewater using a validated analytical method and reveals spatial heterogeneity in xylazine prevalence across sewersheds and its association with elevated fentanyl use. The findings highlight the need for continued surveillance in El Paso and similar communities experiencing an elevated drug overdose burden. Proactive, data-driven strategies informed by wastewater analysis can help guide the allocation of resources and implementation of harm reduction measures. Moreover, this approach can serve as a tool to evaluate the effectiveness of policies and interventions aimed at addressing the xylazine and opioid crisis.[61] By tracking changes in drug use patterns over time, communities can assess the impact of their efforts and make data-driven adjustments to their strategies.

■ ASSOCIATED CONTENT

Data Availability Statement

All data analyzed in this study are included within the article and its Supporting Information. The analysis scripts will be made publicly available on GitHub upon publication.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.5c01016.

Wastewater sample collection information and served population sizes (Table S1), LC-MS/MS ionization and fragmentation conditions (Table S2), LC-MS/MS instrument parameters (Table S3), representative chromatograms demonstrating limits of detection and quantification for xylazine and norfentanyl (Figures S1−S2), and calibration curves for analyte quantification (Figure S3) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Fuqing Wu − *Department of Environmental and Occupational Health Sciences and Department of Epidemiology, University of Texas Health Science Center at Houston School of Public Health, Houston, Texas 77030, United States;* orcid.org/0000-0002-2820-3550; Email: fuqing.wu@uth.tmc.edu

Authors

Katherine M. Joseph − *Department of Environmental and Occupational Health Sciences, University of Texas Health Science Center at Houston School of Public Health, Houston, Texas 77030, United States*

Xuan Qin − *Center for Drug Discovery, Department of Pathology and Immunology, Baylor College of Medicine, Houston, Texas 77030, United States;* orcid.org/0000-0003-3728-5911

Dhvani Parikh − *Department of Epidemiology, University of Texas Health Science Center at Houston School of Public Health, Houston, Texas 77030, United States*

Feng Li − *Center for Drug Discovery, Department of Pathology and Immunology, Baylor College of Medicine, Houston, Texas 77030, United States*

John Balliew − *El Paso Water Utility, El Paso, Texas 79925, United States*

Kristina D. Mena − *Department of Environmental and Occupational Health Sciences and Department of Epidemiology, University of Texas Health Science Center at Houston School of Public Health, Houston, Texas 77030, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsestwater.5c01016

Author Contributions

CRediT: **Katherine Joseph** data curation, formal analysis, investigation, methodology, validation, visualization, writing - original draft, writing - review & editing; **Xuan Qin** formal analysis, methodology, validation, writing - review & editing; **Dhvani Parikh** investigation, writing - review & editing; **Feng Li** resources, supervision, writing - review & editing; **John E. Balliew** resources, writing - review & editing; **Kristina Dawn Mena** resources, writing - review & editing; **Fuqing Wu** conceptualization, formal analysis, funding acquisition, investigation, methodology, supervision, visualization, writing - original draft, writing - review & editing.

Notes

The authors declare no competing financial interest.

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Supporting Information

First Detection of Xylazine in Texas Wastewater and Its Association with Fentanyl Use

Katherine M. Joseph[1], Xuan Qin[2], Dhvani Parikh[3], Feng Li[2],
John Balliew[4], Kristina D. Mena[1,3], Fuqing Wu[1,3*]

Affiliations:

1: Department of Environmental and Occupational Health Sciences, University of Texas Health Science Center at Houston School of Public Health, Houston, TX 77030, USA.

2: Center for Drug Discovery, Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA

3: Department of Epidemiology, University of Texas Health Science Center at Houston School of Public Health, Houston, TX 77030, USA.

4: El Paso Water Utility, El Paso, TX 79925, United States

* Email: fuqing.wu@uth.tmc.edu (FW)

Supplemental Tables and Figures

Table S1. Wastewater sample information and serving population size in El Paso, Texas.

WWTP	Number of Raw Samples Analyzed	Serving Population Size
I	31	98,505
II	31	125,235
III	31	127,250
IV	31	399,424

Table S2: Ionization and fragmentation conditions of xylazine and norfentanyl screened.

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Source Fragmentation	RF Lens (V)	Retention time (min)
Norfentanyl	233.162	84.208	18.31	22.449	115	3.52
Xylazine	221.15	90.125	22.69	34.694	155	3.55
Fentanyl-d5 (IS)	342.288	188.155	23.87	32.653	159	3.62
Xylazine-d6 (IS)	227.15	90.125	23.58	34.694	158	3.55

Table S3. LC-MS/MS instrument parameters for all testing.

Parameter	Setting
Instrument	Thermo Vanquish UHPLC coupled with Thermo TSQ Quantis MS/MS
Column	Agilent XDB-C18 (4.6 mm × 50 mm, 3 μm)
Mobile phase A (aqueous)	Water + 0.1% formic acid (v/v)
Mobile phase B (organic)	Acetonitrile + 0.1% formic acid (v/v)
Gradient	15% to 95% mobile phase B over 6 minutes
Flow rate	0.3 mL/min
Column temperature	40°C
Injection volume	5 μL
Ionization mode	Positive electrospray ionization (ESI+)
Ion spray voltage	3500 V
Sheath gas	Ultra-high purity nitrogen (50 arbitrary units)
Auxiliary gas	Ultra-high purity nitrogen (10 arbitrary units)
Sweep gas	Ultra-high purity nitrogen (1 arbitrary unit)
Collision gas	Ultra-high purity argon
Ion transfer tube temperature	325°C
Vaporizer temperature	350°C
Acquisition mode	Selected Reaction Monitoring (SRM)
Acquisition window	1.5-5.5 min

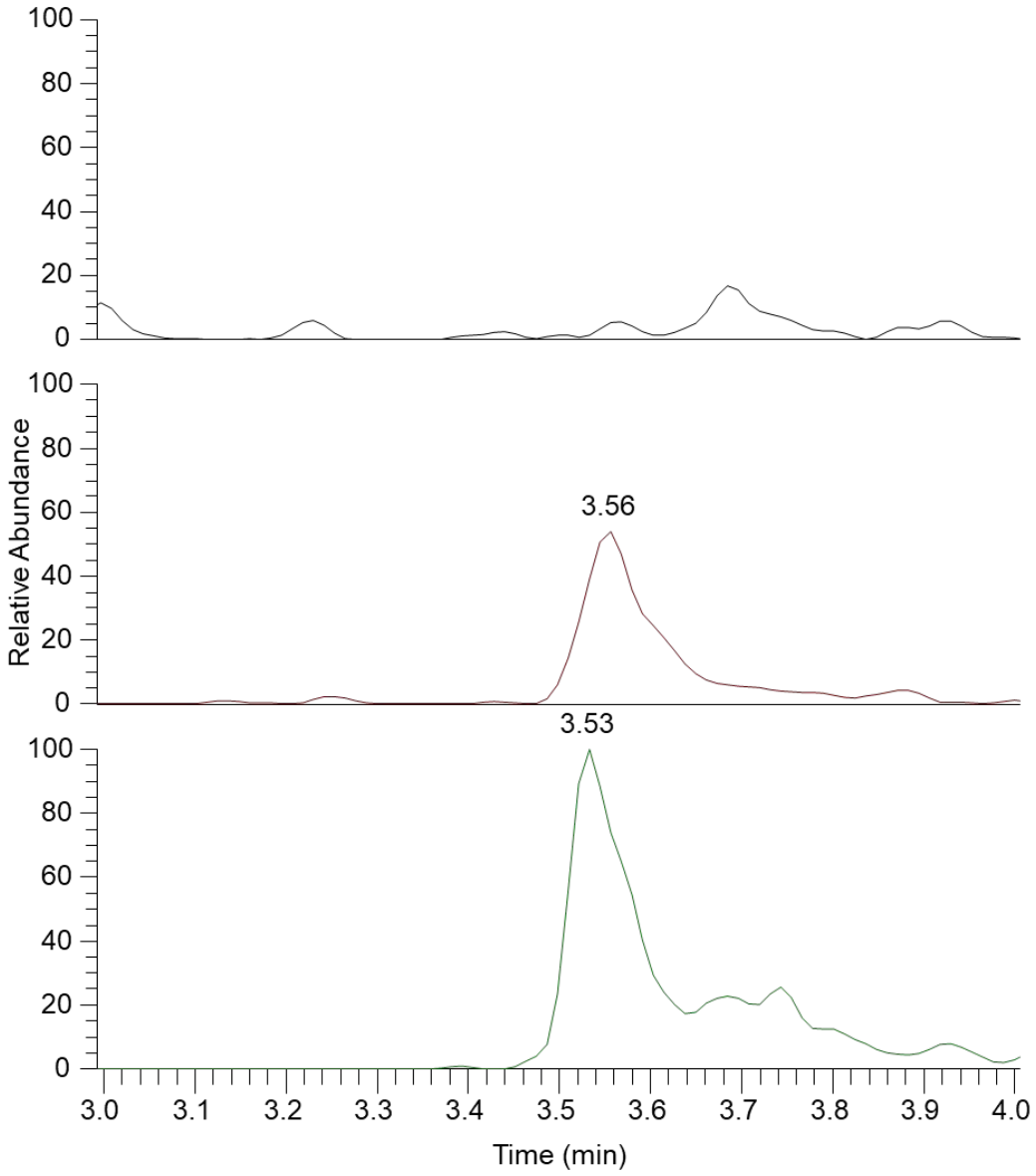

Figure S1. LC-MS/MS chromatograms for Xylazine, LOD, and LLOQ. Top: Blank sample (HPLC-grade water) showing absence of interference at the xylazine retention time. Middle: LOD (0.1 ng/L). Bottom: LLOQ (0.2 ng/L). The peak heights were normalized globally across the panels.

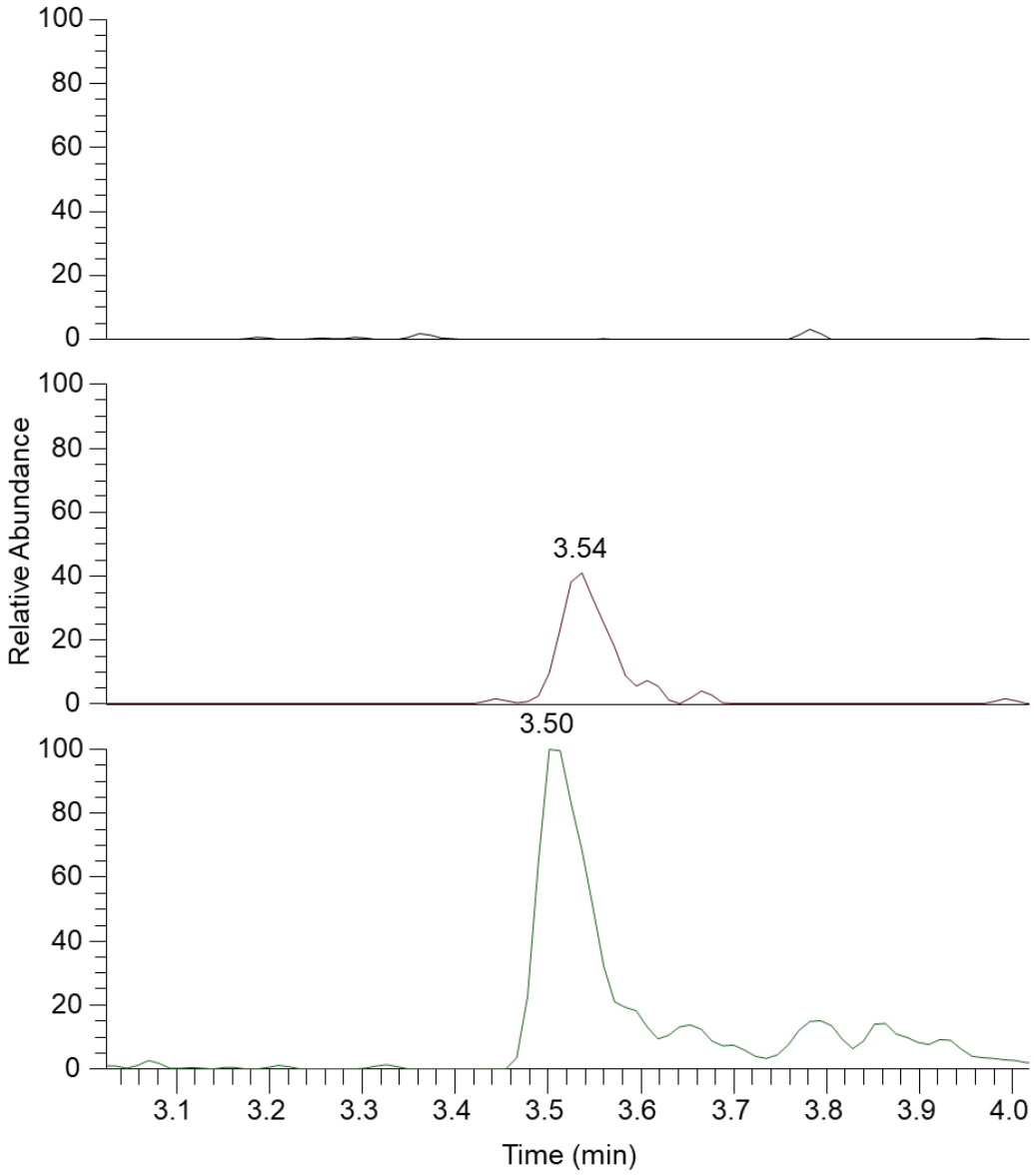

Figure S2. LC-MS/MS chromatograms for Norfentanyl, LOD, and LLOQ. Top: Blank sample (HPLC-grade water) showing absence of interference at the norfentanyl retention time. Middle: LOD (0.5 ng/L). Bottom: LLOQ (1 ng/L). The peak heights were normalized globally across the panels.

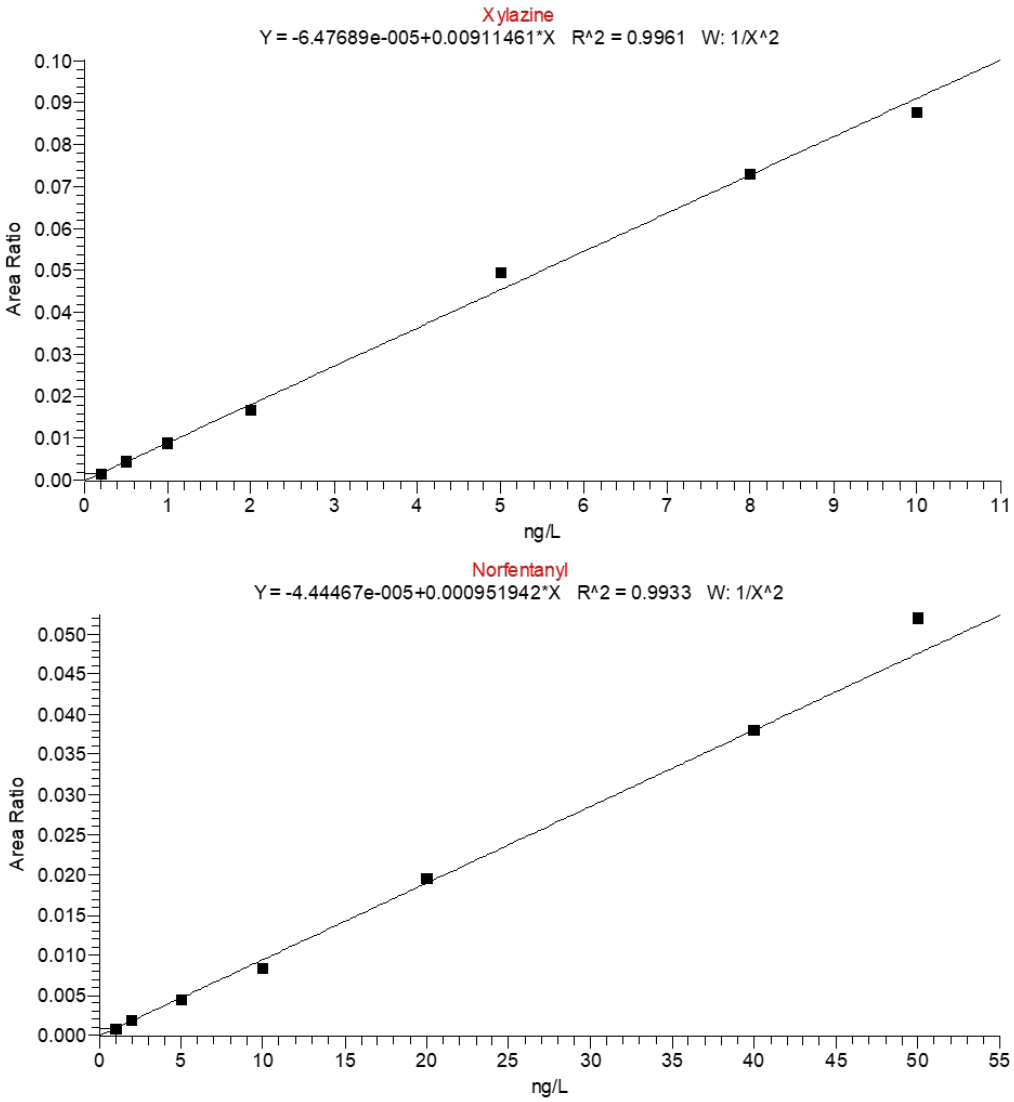

Figure S3. Representative standard curves for xylazine and norfentanyl quantification. Top: Standard curve showing the relationship between xylazine concentrations in water before SPE extraction (ng/L) and peak area ratio (xylazine/IS) across the analytical range of 0.1-10 ng/L, with R^2 0.9961. Bottom: Standard curve showing the relationship between norfentanyl concentrations in water before SPE extraction (0.1-50 ng/L) and peak area ratio, with R^2 0.9933.