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RESEARCH ARTICLE

Wastewater Surveillance for Xylazine in Kentucky

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Introduction: In the U.S., xylazine, the veterinary non-opioid sedative, has emerged as a major threat to people who use illicitly manufactured fentanyl and other drugs. The aim of this study was to compare wastewater detection of xylazine with other public health and safety surveillance data from 2019 to 2023 in Kentucky.

Methods: Wastewater samples from 5 rest areas, 2 truck weigh stations, and 4 wastewater treatment plants were tested for xylazine. Wastewater xylazine positivity rates were compared with xylazine-positive submission rates from the National Forensic Laboratory Information System and Kentucky's fatal overdoses in 6-month periods (Period 1=January−June; Period 2=July−December).

Results: Xylazine was detected in 61.6% (424 of 688) of daily wastewater samples from roadway sites/wastewater treatment plants. For roadways, detection increased from 55% (Period 1, 2021) to 94% (Period 1, 2023), and wastewater treatment plants had an overall detection of 25.8% (n=66 samples, Periods 1 and 2, 2022). Increasing roadway positivity corresponded to trends in National Forensic Laboratory Information System xylazine-positive submission rates: from 0.19 per 1,000 submissions (Period 1, 2019) to 2.9 per 1,000 (Period 2, 2022, latest available). No deaths from xylazine were reported publicly in Kentucky, although this study's authors identified 1−4 deaths (true count suppressed) in the overdose surveillance system, which, in back-of-the-envelope comparisons with other states, is far fewer than expected.

Conclusions: Wastewater signals indicate broad geographic exposure to xylazine in Kentucky, yet health outcomes data suggest otherwise. These findings may inform regional, national, and international efforts to incorporate wastewater-based drug surveillance. Harm-reduction activities along roadways and other suitable locations may be needed.

INTRODUCTION

In the U.S., fatal overdoses involving xylazine, a veterinary non-opioid sedative, exponentially increased from 2018 to 2021.[1,2] The underlying reasons for the rapid reintroduction of xylazine into the drug supply are unclear, but these overdoses almost always co-occur with illicitly manufactured fentanyl in jurisdictions reporting toxicology testing for drug-involved deaths.[3−6] Analysis of surveillance data using medicolegal death investigation or driving under the influence samples

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2773-0654/$36.00
https://doi.org/10.1016/j.focus.2024.100203

suggests that xylazine primarily emerged in the eastern U.S. and spread westward thereafter.[4,7] In April 2023, xylazine was officially designated as an emerging threat by the Office of National Drug Control Policy.[8]

The state of Kentucky, especially its eastern, Appalachian region counties, is vulnerable to negative outcomes associated with drug use.[9] The state has been heavily impacted by the opioid crisis, including outbreaks involving novel psychoactive substances.[10−14] Thus, it is somewhat surprising that, as of June 2021, Kacinko et al.[7] tested for but did not detect xylazine in medicolegal death investigation/driving under the influence samples collected from Kentucky even though all 7 of its bordering states had at least 1 positive case. To the east, Kentucky borders 2 states in the multistate region (including Pennsylvania) with the highest age-adjusted rate of drug overdoses involving xylazine.[15] From healthcare settings, the presence of xylazine (no further details available) was only first publicly reported in Kentucky sometime between April 2021 and March 2022.[16] In neighboring West Virginia, a state with a similar demographic profile, there were 117 xylazine-involved deaths from January 2019 to June 2021, with >90% occurring in 2020 and 2021.[5] To date, no xylazine-involved deaths have been publicly reported in Kentucky.

Yet, xylazine was detected in raw wastewater samples from 2 Kentucky interstate welcome centers/rest areas (rest areas) and 1 commercial truck servicing facility (weigh station) between September and December 2021.[17] Wastewater surveillance for opioids and novel psychoactive substances is a growing public health strategy globally, and multiple studies have used wastewater detection to identify drug use profiles in communities and locations in Kentucky.[18−20]

The purpose of this study is to examine monthly results from expanded wastewater surveillance for xylazine from 5 rest areas, 2 weigh stations, and 4 wastewater treatment plants (WWTPs) from January 2019 to February 2023 in Kentucky. Furthermore, authors triangulate and contextualize the wastewater samples with xylazine-positive law enforcement submissions to the National Forensic Laboratory Information System (NFLIS), the Kentucky State Police Central Forensic Laboratory, and fatal overdoses from Kentucky's Drug Overdose Surveillance System.

METHODS

Study Sample

Detailed data collection, sample preparation/handling, and xylazine quantification in roadway wastewater samples were previously reported and made available as Appendix Material (available online).[17] Briefly, roadway sampling began in 2 rest areas in western Kentucky in September 2021, and the other sites (3 rest areas and 2 weigh stations) had staggered start dates thereafter (Appendix Figure 1, available online). Composite samples were collected over a 24-hour period, and 500 ml were transferred to bottles for freezer storage. For a 7-day sampling week, 7 total samples would represent the denominator for the analysis. A total of 622 samples (482 from rest areas and 140 from weigh stations) were collected from roadway sites during the study period.

The roadway sampling sites were largely chosen for the convenience of the sampling team (e.g., reasonable drive times), state border locations, and stimulant-related county-level factors (e.g., prescribing rates) owing to the original stimulant epidemiologic focus of this project. The 4 WWTPs were serendipitously added through a collaboration with a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) wastewater surveillance project.

In a separate pilot project, 4 WWTPs in Eastern Kentucky were recruited for once-a-week (Wednesday) sampling for a 3−6-month period limited to February −August 2022. The populations served by these WWTPs ranged in size from <5,000 to 50,000 people. Composite samples were collected over a 24-hour period from the influent wastewater using each WWTP's existing sampling protocol. Sample aliquots of 250 ml were transferred to the laboratory in sterile, high-density polyethylene bottles and kept on ice to maintain a ∼4°C sample temperature. Upon laboratory arrival, 50ml aliquots of each sample were stored in −80°C freezer until further processing. A total of 58 samples were collected from WWTPs. All wastewater samples were chemically analyzed using a validated isotope dilution mass spectrometric method at Murray State University. The authors report positive samples as those with concentrations higher than the limit of detection.

Measures

Study authors downloaded NFLIS data from the U.S. Drug Enforcement Administration's publicly accessible web-based query system.[21] NFLIS data for Kentucky are submitted by the Kentucky State Police Forensic Laboratories for testing performed in their laboratories on submissions of drugs seized and purchased from state and local law enforcement agencies. NFLIS does not provide county-level data, so the information presented by county was requested directly from the state laboratories. To standardize xylazine-positive submission counts, the authors calculated the rate of xylazine detected per 1,000 submissions of total samples of seized/purchased drugs. Counts of xylazine-involved deaths were provided by the Kentucky Injury Prevention Research Center. The authors

aggregated results to the publicly available NFLIS reporting periods (8 in total) of each year beginning in 2019: January–June (Period 1) and July–December (Period 2).

County classifications were downloaded from the Appalachian Regional Commission and the U.S. Drug Enforcement Administration's High Intensity Drug Trafficking Areas (HIDTA) program.[22,23] Briefly, HIDTA county designation indicates that an area is a significant center of illegal drug production, manufacturing, importation, or distribution. This study was exempted by the University of Kentucky's IRB (#59087).

RESULTS

Wastewater (All Samples)

During the study period, xylazine was detected in 62% (424 of 688) of all wastewater samples either from roadway sites or WWTPs. The prevalence of positive samples by data source and aggregated to 6-month periods are provided in Figure 1. A map of sampling sites and counties where xylazine was detected in Kentucky is provided in Figure 2. Table 1 provides sample counts and positivity rates for all wastewater sites in the study.

Roadway

In Period 2 of 2021, 54% (61 of 112 samples) of roadway samples tested positive for xylazine, decreasing to 38% (60 of 159) in Period 1 of 2022 before doubling to 78% (184 of 237) in Period 2 of 2022. In Period 1 of 2023, overall roadway detection increased again to its maximum of 90% (102 of 114 samples), with 94% (81 of 86 samples) and 75% (21 of 28 samples) detection for rest areas and weigh stations, respectively.

Wastewater Treatment Plants

Overall, WWTP samples from Eastern Kentucky had a 25.8% xylazine detection rate (16 of 57 samples, February–August 2022). Period 1 of 2022 had a 28% positivity rate, whereas Period 2 had an 11.1% positivity rate.

Law Enforcement Submissions

National Forensic Laboratory Information System. For periods with data aligned to the roadway samples, the detection rate of xylazine from Kentucky's NFLIS samples was 3.9, 1.6, and 2.9 per 1,000 submissions, respectively.[21]

State Laboratory. The state laboratory reported 22 counties (18%) in Kentucky with at least 1 sample positive for xylazine. Montgomery County had at least 1 positive submission in 7 of 8 periods, followed by Pike County and Mason County with 5 of 8. Most counties had <3 positive submissions for any given period, with the exceptions of Boyd, Fayette, and Mason, which had 6 (Period 1, 2020), 8 (Period 2, 2019), and 17 (Period 1, 2022), respectively. Every county with at least 1 positive submission was located in the Eastern/Central part of the state (14 Appalachian counties, 6 with HIDTA designations), with the exception of McCracken County in the Western part of Kentucky. McCracken County is home to 1 of the roadway sampling sites.

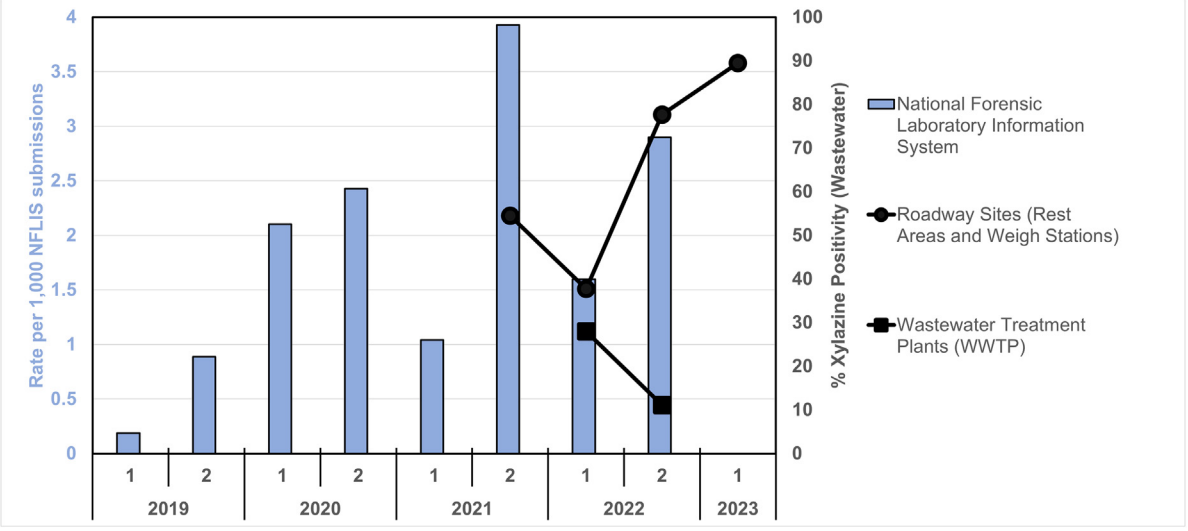

Figure 1. Xylazine detection (positivity rate) in wastewater samples from roadways (circles), WWTPs (squares), and submissions to the National Forensic Laboratory Information System (dark blue bars), 2019–2023, by 6-month periods in Kentucky.
1=January–June; 2=July–December. Roadway sampling occurred between September 2021 and May 2023, and WWTP sampling includes February to June (Period 1) and July and August (Period 2) of 2022.

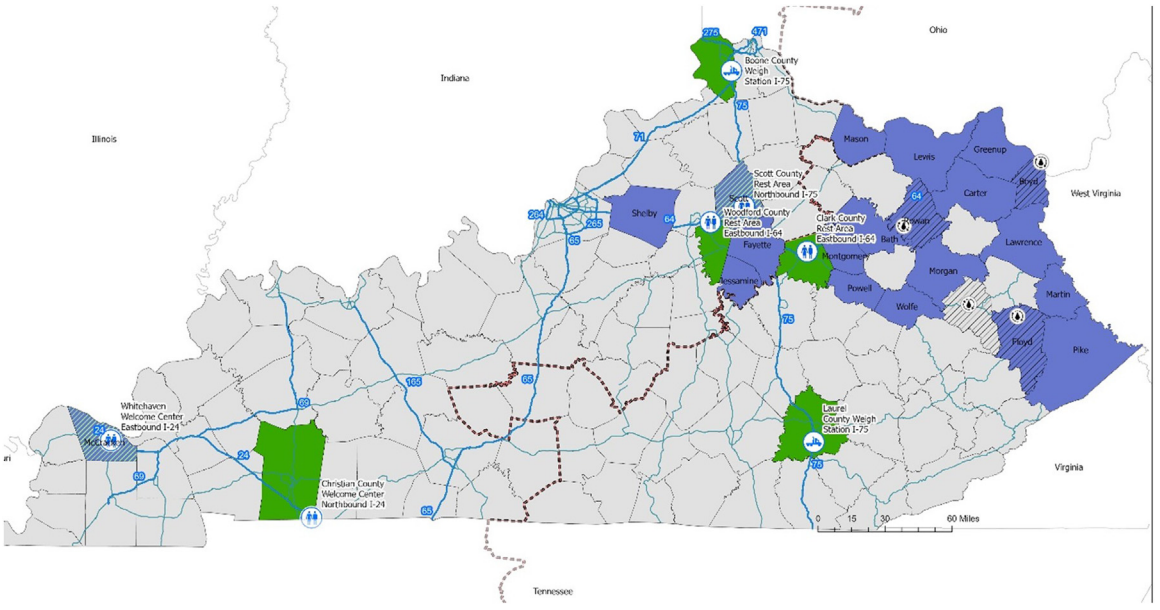

Figure 2. Map of sampling sites for this study: rest areas, weigh stations, and WWTP sites in Kentucky.
Blue-shaded counties are those with 1 or more samples from the state forensic laboratory positive for xylazine over the study period (hashed lines indicate that a WWTP in the county also had 1 or more samples positive for xylazine). Green-shaded counties show where roadway samples were taken (hashed lines show where state forensic laboratory submissions were positive for xylazine). Traversing dotted lined defines Appalachian counties to the east.
WWTP, wastewater treatment plant.

Fatal Overdoses. The most frequently used postmortem toxicology panel conducted on suspected drug overdoses in Kentucky during the study period did not include xylazine, resulting in <10% of suspected overdose decedents being tested. Among those decedents who received this panel, there were 1−4 xylazine-involved fatal overdoses over the study period. Additional information is not available owing to state data suppression preferences that require counts that are <5 (except zero) to be suppressed to avoid a potential breach of confidentiality.

DISCUSSION

In Kentucky, evidence indicates that xylazine is circulating in populations using interstate rest areas, commercial truck weigh stations, and municipal WWTPs. Furthermore, this study found that xylazine was present in drug exhibits seized by federal/state law enforcement, a finding, to the authors' knowledge, not previously reported publicly in Kentucky. For xylazine, the observed trends in roadway wastewater samples (i.e., brief decline in Period 1 of 2022 followed by a doubling of the detection rate) corresponded with a similar trend in NFLIS positivity rates at the state level. Although data points to compare are limited, this is an important potential correlation because NFLIS submissions have previously been associated with fatal overdoses in Kentucky and other states in the region.[24,25] For example, in Philadelphia, the national epicenter of xylazine overdoses, approximately 23% (n=263) of all overdose deaths were positive for xylazine in 2019.[4] In 2019, NFLIS submissions positive for xylazine in the presence of heroin and fentanyl also peaked at 25% in Pennsylvania.[26] The present study's authors calculated that the xylazine submission rate from July to December 2019 (Period 2, 2019) in Pennsylvania hit a maximum of 14 per 1,000 submissions, nearly 14 times higher than in Kentucky. Unpublished wastewater data in the Philadelphia area are also registering xylazine concentrations that are several orders of magnitude higher than other city comparators (personal communication, Sabo-Attwood). As of July 2023, xylazine is almost as prevalent as heroin in law enforcement submissions that are also positive for fentanyl (22% vs 29%, respectively) and has been more prevalent than cocaine since 2020.[27] The concordance and intensity of these indicators speak the value of triangulating multiple data sources.

Xylazine-involved fatal overdoses have occurred in Kentucky, but routine and comprehensive testing for xylazine was not conducted during this time period. Obtaining exact overdose counts is hampered by a state data suppression policy, which may itself hinder rapid response.[28] Therefore, the authors of the present study

Table 1. Xylazine Testing Results From Roadway Sites (Rest Areas and Weigh Stations), WWTPs, and the NFLIS by 6-Month Periods in Kentucky, 2019–2023

Sample type	P	Year	Month	Positive for xylazine	Total samples or submissions	% positive or rate per 1,000
Rest areas (*n*=5)	P2	2021	September–December	50	91	55%
	P1	2022	January–June	50	136	37%
	P2	2022	July–December	137	169	81%
	P1	2023	January–May[a]	81	86	94%
	Rest area total			318	482	66%
Weigh stations (*n*=2)	P2	2021	September–December	11	21	52%
	P1	2022	January–June	10	23	43%
	P2	2022	July–December	47	68	69%
	P1	2023	January–May	21	28	75%
	Weigh station total			89	140	64%
Roadway (rest areas + weigh stations; *n*=7)	P2	2021	September–December	61	112	54.5%
	P1	2022	January–June	60	159	37.7%
	P2	2022	July–December	184	237	77.6%
	P1	2023	January–May	102	114	89.5%
	Roadway total			407	622	65.4%
WWTP, *n*=4	P1	2022	February–June	16	57	28.0%
	P2	2022	July–December	1	9	11.1%
	WWTP total			17	66	25.8%
NFLIS	P1	2019	January–June	2	10,721	0.19
	P2	2019	July–December	11	12,396	0.89
	P1	2020	January–June	24	11,424	2.10
	P2	2020	July–December	25	10,299	2.43
	P1	2021	January–June	46	14,389	3.20
	P2	2021	July–December	15	11,712	1.28
	P1	2022	January–June	19	11,889	1.60
	P2	2022	July–December	32	11,048	2.90
	NFLIS total			174	93,878	1.85

[a]Not a complete 6-month period.
NFLIS, National Forensic Laboratory Information System; P, period; WWTP, wastewater treatment plant.

argue that xylazine involvement in drug overdose mortality is likely undercounted, so they estimated the potential magnitude of xylazine-involved overdose deaths in Kentucky using data reported from other states.

The annual percentage of xylazine-involved overdose deaths at the state level has ranged from 0% (North Carolina, 2020), to 4% in West Virginia (2019 to mid-2021), and to 19.3% (Maryland, 2021) of all overdoses.[4,5] If a 2% expected rate is conservatively applied to Kentucky's 3,281 drug overdose deaths (for 2019 and 2020), approximately 65 deaths involving xylazine would be expected.[29] The percentage of overdose deaths involving illicitly manufactured fentanyl with xylazine detected is higher: ranging from 0% (3 states) to 28% (Maryland) in 31 states examined (excluding Kentucky), with an overall detection rate of 9%.[2] Applying the 9% overall rate, approximately 188 deaths would be expected (9% × 2,097 fentanyl deaths for 2019 and 2020). Thus, given this study's triangulated surveillance signals, authors argue that the reported xylazine-involved deaths are far fewer than expected. This data gap is also reflected in the estimation of xylazine involvement in nonfatal overdoses treated in emergency departments where <40% of suspected overdoses receive any form of toxicology screening or confirmatory testing much less with a panel that includes xylazine.[30] Future work examining the unique characteristics of xylazine exposure symptoms, such as necrotic skin infections, may provide additional clues as to its impact on nonfatal health outcomes.[31]

The absence of identified xylazine involvement in fatal overdoses may be a function of other components of surveillance bias (e.g., coroners not documenting the presence of xylazine) but may also indicate that xylazine in the drug supply is not causing additional mortality.

Inconsistencies in detecting xylazine in the drug supply compared with NFLIS and overdose investigations are not surprising. Friedman and colleagues[4] noted that toxicological and drug testing in North Carolina found xylazine in street-acquired samples even though no xylazine-involved deaths had been reported in the state. More specifically, these samples from 30 counties in North Carolina yielded a 27% positivity rate (153 of 574).[32] Similar to Kentucky, NFLIS data for North Carolina show an apex (n=37) in xylazine-positive submissions in Period 2 of 2021.

The broad geographic spread of xylazine-positive samples in Kentucky suggests likely exposure throughout the state that is not apparent from fatal and nonfatal drug overdose data alone. Intentional triangulation of multiple surveillance systems is critical for monitoring the evolving opioid and drug crisis.[33–35] The city of Tempe, Arizona, a potential model, monitors multiple opioid types and other biomarkers and makes results publicly available on a weekly basis at subcity geographic resolution.[36]

Wastewater surveillance of drugs is an integrated and routinized component of epidemiologic surveillance in multiple countries, including those in the European Union and Australia.[37–39] Although studies correlating health outcomes with wastewater surveillance are still relatively rare, some systems are maturing rapidly to allow for actionable insight into populations and policy. For example, in South Australia, fentanyl use (population and dose adjusted) appears to be declining from February 2019 to October 2022. This trend observed in wastewater appears correlated with a sustained decline in fatal synthetic opioid overdoses reported in the region.[40,41] In Mexico, fentanyl and its metabolite norfentanyl were surprisingly detected in wastewater treatment facilities in 2 of 7 cities studied between November 2017 and February 2018. Although these quantitative signals were arguably weak, the wastewater identification of fentanyl predated more recent research identifying fentanyl in the drug supply, counterfeit pills, and previously untested decedents in Mexico.[42–44] In the context of roadways, the percentage change in WWTP-measured methamphetamine increased significantly over a 61-month period, and this change was linearly correlated with oral swab samples from drivers taken at random road stops in Australia.[45] In addition to examining illicit drugs using wastewater, a study in Connecticut found an increase in hydroxychloroquine in wastewater the week after emergency use authorization in the early coronavirus disease 2019 (COVID-19) period.[46] There are multiple methodologic limitations that drive variability in wastewater surveillance of drugs that need to be addressed, but the U.S. Centers for Disease Control and Prevention National Wastewater Surveillance System for COVID-19 is a model for large-scale surveillance that could be potentially adopted and repurposed for drug targets.[18,47–49]

In addition to drug surveillance opportunities, rest areas and trucking weigh stations have a new role to play in harm-reduction strategies from a public health perspective. In September 2023, the governor of Ohio announced that naloxone, the opioid reversal medication (which is measurable in wastewater[50]), will be made available in 65 rest areas in the state.[51] Furthermore, xylazine test strips are now available, and qualitative data suggests that people using drugs are amenable to using them.[52,53] This study's findings indicate that roadway facilities may be viable distribution points for test strips.

Limitations

This study cannot quantify the proportion of Kentucky residents using roadway facilities, so these results may reflect mobile, nonresident populations. The authors cannot rule out that passengers, staff on site, and other community members may be using drugs and the roadway bathroom facilities. Commercial trucks often use general commuter rest areas, but the reverse is less likely due to stricter entry controls to weigh stations. It is possible that xylazine originates from veterinary sources in WWTPs, but this seems highly unlikely in the roadway samples that are essentially taken at the point of immediate discharge. The sampling days were not randomly selected during the month, and authors deliberately sampled holidays/special occasions (data not shown) known to have higher drug use. Population drug consumption rates (mass loads) based on the concentration of xylazine in wastewater samples are preferred to the qualitative (yes/no) detection frequencies reported in this paper. However, these calculations are challenging, especially for xylazine, because human excretion and metabolism studies required for estimating consumption fractions are lacking. Law enforcement submissions may reflect operational hot spots and are not representative of all counties.

CONCLUSIONS

This work can inform the Office of National Drug Control Policy's response plan[8] to enhance epidemiologic systems, including wastewater testing for drugs, in the U.S.

ACKNOWLEDGMENTS

This project was supported by Award #2020-R2-CX-0013, awarded by the National Institute of Justice, Office of Justice

Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice. Additional support provided by the NIH grants 1U01DA053903-01 and pilot funds from the University of Kentucky's Substance Use Priority Research Area (SUPRA), supported by the Vice President for Research. The authors acknowledge the following: Jeremy Triplett of the Kentucky State Police Central Forensic Laboratory, the Jones/Ross Research Center, Chemistry Department, Murray State University for providing access to the UPLC-MS/MS, David Cornett (Kentucky Transportation Cabinet), Sharon Lovejoy, Benton Hoagland, James Owen, Derek Petrey, Larry Bailey, and Tony Hicks for assistance in sampling access at the rest areas, and Anna Stites, Katherine Gray, Tyler Whitt, Andrew Windhorst, Landon Jones, and DeMarkus for sample analysis/data preparation. Declaration of interest: none.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.focus.2024.100203.

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