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FINAL RESEARCH REPORT

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Project Title

Wastewater Epidemiology To Examine Stimulant Trends (WeTEST)

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TABLE OF CONTENTS

Project Summary 3
Research Questions 4
Methods 5
Sampling Plan 5
Analytical Chemistry 10
Comparative Analysis 12
Expected Applicability of the Research 14
Participants and Other Collaborating Organizations 14
Changes in Approach 14
Outcomes 15
Results and Findings 15
Limitations 34
Artifacts 34
List of Products 34
Datasets Generated 34
Dissemination Activities 34
Aknowledgements 35
References 37

PROJECT SUMMARY

Drug overdose mortality, particularly involving psychostimulants such as cocaine and methamphetamine, has become a significant public health crisis in the United States.[1–5] Kentucky (KY), a state already severely affected by the opioid epidemic,[6] has experienced alarming rates of fatal drug overdoses, with stimulant use contributing increasingly to these fatalities.[7] In 2018, KY reported 439 stimulant-involved overdose deaths, with a rate of 10.6 per 100,000.[7] By 2022, this number surged to 1,113, with a rate of 26.5 per 100,000 population—a 153.5% increase.[7] This sharp increase has compounded the state's existing public health burden associated with drug misuse.[8] Moreover, KY's counties exhibit significant variability in demographic, economic, and healthcare access characteristics,[9,10] which are key determinants influencing the rates of drug overdose mortality.

New drug surveillance methods are needed to enhance the speed and accuracy of identifying substances affecting U.S. communities, including those in KY. Wastewater-based epidemiology (WBE) is a novel, yet established, scientific approach that offers a robust, non-invasive, and near real-time method for detecting population drug exposures. By analyzing trace drug residues in untreated wastewater, WBE provides a comprehensive tool for ongoing surveillance and early warning of drug exposure.[11] Our project, "Wastewater Epidemiology To Examine Stimulant Trends" (**WeTEST**), used WBE to develop a temporary wastewater surveillance system targeting select traffic rest areas and truck weigh stations along three interstate highways passing through KY. During the project period, we partnered with the University of Kentucky's Substance Use Priority Research Area (SUPRA) to obtain additional wastewater samples from four wastewater treatment facilities (WWTF) in the eastern part of KY (i.e., Appalachian area). Thus, our project offers insights into stimulant use patterns and drug use trends among populations utilizing these select rest areas, truck weigh stations, and WWTFs. The **major goals** of this project, as described in the original proposal, include the following:

1. Build a robust, temporary wastewater surveillance system along traffic rest areas and facilities servicing truck drivers (i.e., weigh stations) along select interstate highways in KY.
2. Examine stimulant use trends in the populations using these facilities and traversing "hot spot" areas of the state.
3. Compare rest area wastewater results to a municipal water system (i.e., WWTF) to examine the drug profile variability between roadways and community use.
4. Compare wastewater results to other population-based drug use indicators (i.e., stimulants dispensed according to KY's Prescription Drug Monitoring Program [PDMP]).
5. Establish a unique collaboration between the Appalachian High Intensity Drug Trafficking Area (HIDTA), KY Transportation Cabinet, KY Cabinet for Health and Family Services (KY CHFS), Murray State University (MSU), and the University of Kentucky (UK).

Research Questions

This project used WBE to investigate multiple research questions related to psychostimulant use (including, but not limited to, cocaine, amphetamine, and methamphetamine) and their variability along roadway facilities in KY. **Research questions**, as originally described, include the following:

1. What are the stimulant drug types detected in the general driving population along three major interstate highways (I-24, I-64, and I-75) in KY?
2. Are there any novel stimulant drug types detected during the project study period?
3. Do stimulant drug types align with documented stimulant drug problems in communities where the facilities are located?
4. Does stimulant drug prevalence in facilities correlate with the other reported public health and safety indicators such as road accidents, associated drug crime and/or controlled substance prescribing patterns?

5. What are the stimulant drug types used by truck drivers along interstate trucking facilities (i.e., weigh stations)?
6. Are stimulant drug types different between truck drivers and the general population?
7. Do stimulant drug types differ based on traffic entering/leaving the state along the Tennessee (TN) border? Are stimulant drug types different based on travel direction?
8. Do interstate stimulant drug types correlate with drug detected from a nearby municipal WWTFs?
9. Are there daily, monthly, and seasonal variations in stimulant drug types in the general population and/or truck drivers?

Methods

Sampling Plan

Facility types. Wastewater was sampled from five rest areas—consisting of two "Welcome Centers" and three rest areas—and two commercial truck service facilities (i.e., weigh stations) along major interstate highways in KY. Images of the selected facilities are shown in **Figure 1** and aerial photographs are shown in **Figure 2**. Welcome Centers typically offer restroom facilities as well as expanded tourism information. The Christian County Welcome Center is located on the TN border. Trucking weigh stations offer restroom facilities to the truck driving population as well as cargo weighing and inspection services. We obtained permission from the KY Transportation Cabinet to conduct this research who arranged access to the roadway facilities. This arrangement involved gaining access to manholes, water meters, and permission to install people counters on doorway entrances to the bathrooms. **None of the data WeTEST collected was identifiable.**

Figure 1. Photographs of the Christian County Welcome Center, the Clark County Rest Area, and the Laurel County Trucking Weigh Station.

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Figure 2. Aerial photographs of **WeTEST** roadway facilities. See labels in each photograph for site name.

Site selection. We used multiple criteria to select the original roadway sites. First, each site had to be accessible to the two universities involved in the project and close enough to have reasonable drive times for staff to collect samples. The plan was for staff to drive to their designated rest areas and return the samples to the laboratory refrigerators at each university on the day they were collected. Second, we wanted variability in drug arrest rates as an indicator of general drug-related activity in the counties where the facilities are located. The idea was to determine if wastewater drugs were indicative in both type and amount in broad terms of low and high drug activity counties.

We learned that the drive times became onerous for UK staff supporting the project, so we changed the strategy by a) enlisting the support of truck weigh station staff already working at the Laurel County Truck Haven (the furthest site from UK) and b) hired a staff member solely dedicated to collecting samples from multiple facilities locations near UK. When possible, we stored the samplers on-site during non-sampling periods to avoid having staff transport the sampling machines multiple times each month. Sampling for sites in the MSU collection zone were handled by Dr. Subedi and his team.

Sampling days. The ideal sampling plan was designed as seven consecutive days (to include weekend samples), every month, for one year with flexibility to sample during major holidays and/or other special events. The concept was to provide an average drug snapshot of a typical week each month. **Figure 3** shows our actual sampling calendar for all roadway facilities. The project faced COVID-19 related delays so sampling officially stated in September 2021 with three sites (Christian County, McCraken County, Laurel County Truck Haven). After each site started, gaps in continuous sampling and deviations from our ideal plan occurred for various reasons throughout the project including sampling machine breakdowns, rest area closures by state agencies, interstate construction, weather events, and/or personnel availability. Remarkably, we maintained continuous sampling during one of the worst tornado events to hit Western KY (very near to our sampling locations) on December 10-11, 2021.[12] We actually extended the sampling period for Laurel County Truck Haven to 15 months because the truck haven staff appreciated the project and sampling was fairly routine and not overly time consuming for them. **A lesson learned was the value of recruiting on-site staff and the relative ease of training lay people for sampling.**

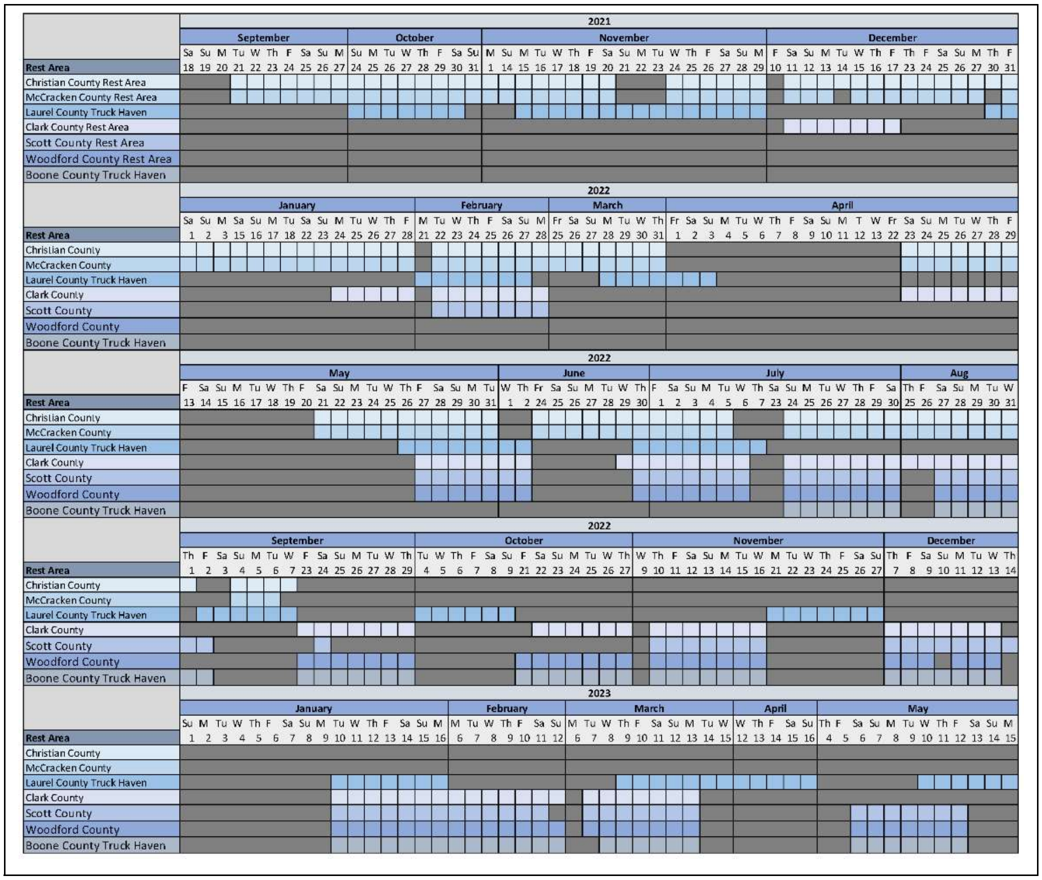

Figure 3. WeTEST sampling calendar for roadway facilities, September 2021 – May 2023. Colored blocks represent days sampled according to facilities listed in rows. Grey periods are days not sampled.

Through a special collaboration at UK (SUPRA grant), four WWTFs were sampled on a weekly basis from February 2022 to August 2022. The specific dates of these samples can be found on our **WeTEST online dashboard available here:** **https://public.tableau.com/app/profile/ipop/viz/WastewaterEpidemiologyToExamineStimulantTrendsweTESTupated012824/Front**.

Collection. For roadways, samples were collected in one liter polypropylene bottles (grab sample) and time stamped. Staff routes were optimized so that samples were put into deep freeze on site or within four hours of collection. For example, refrigerators (-5 degree C) were purchased for the Laurel County Truck Haven given its distance from UK and samples were retrieved once per month. The samples were stored on dry ice while in-route, and at the end of the quarter, they were driven by staff to Dr. Subedi for long-term storage and analysis. Staff responsible for wastewater collection received training in chemical and biohazards safety and proper sampling, storage, and handling. A YouTube video demonstrating the collection process was produced and disseminated via the web for future use.[13]

Table 1 shows the total number of sampling days by site location as counted from the sampling calendar. For various reasons, samples collected were not always acceptable for chemical analysis so the true number of samples analyzed can be found in tables presented in **Figure 5** and differs slightly from the calendar-based sampled day count.

Table 1. Total Sampling Months and Days by Roadway Facility, Type and Interstate Highway

Site	Abbr.	Number of Months	Number of Days
Christian County (RA, I-24 W)	CC	12	116
McCraken County (RA, Whitehaven, I-24 E)	WH	12	113
Laurel County Truck Haven (I-75)	LC	15	91
Clark County (RA, I-64 E)	WC	15	103
Scott County (RA, I-75 N)	GT	12	78
Woodford County (RA, I-64 E)	WF	10	67
Boone County Truck Haven (I-75)	CR	10	69

Analytical Chemistry

The samples were extracted using solid-phase extraction and analyzed using the state-of-art high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) method and briefly described below. For a more detailed description, we refer the reader to the developed and validated analytical methods reported elsewhere.[14–16]

The analysis of samples using HPLC-MS/MS is comprised of rigorous quality assurance and quality control procedures including laboratory control, matrix-spike (and duplicate), ten-point calibration curve at environmentally relevant concentrations, and continuous calibration verification standards. The internal standards (isotope-labeled standard of EACH target drug) were spiked to the samples prior to sample preparation so that any loss of target drugs during sample preparation and instrumental analysis was self-corrected. This method is called isotopic-dilution mass spectrometric analyses.

Ultra-high-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 used 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. Relative retention time (±0.05 min) to their deuterated forms, two parent-to-daughter ion transitions, 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 was optimized for all analytes and internal standards. The source parameters used were 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.

Consumption levels. The concentrations (nanogram per liter) of drug residues (or metabolites to eliminate non-consumed but the directly discharged fraction) was multiplied by the average total wastewater discharged (liters) at the rest area to get total mass of drugs discharged (e.g., “consumption”, “mass load”). The stability of drugs in wastewater for approximately six hours was used to correct the potential loss of drug residues in wastewater prior to the sample preparation.[16] The human excretion rate of drug residues (parent or metabolites) was used to determine the consumed amount of drugs and found in the human health literature and typically reported in wastewater studies. For example, we reported excretion rates for 17 parent or metabolite compounds in supplemental tables in Grey et al. (2022). The total amount of drugs (in milligrams) was then divided by the typical dose of a drug to determine the number of doses of drugs consumed by the restroom visitors along interstate highways and communities served by the WWTFs. Typical doses were taken from Postigo et al. (2008)[17] and Baselt (2014).[18] Details on how mass loads and their confidence intervals are calculated using Monte Carlo analysis to account for uncertainty can be found elsewhere.[19]

Staff responsible for laboratory analysis received comprehensive training in sample storage, analytical method development, method validation, sample extraction, and analysis. All samples were processed and analyzed for stimulants and novel psychoactive substances. **Though not a stimulant drug, during the project period we developed a procedure for identifying xylazine—a veterinary sedative—in samples and reported our findings in the peer-reviewed literature given the high public health interest and emergency declaration from the White House in April 2023.**[18,19]

Novel psychoactive stimulants (NPS). The complete analytical method for the NPS stimulants is detailed in Grey et al. (2020). All high purity NPS standards and internal standards were purchased from Sigma Aldrich (St. Louis, MO) and Cayman Chemical (Ann Arbor, MI). Analytical method to analyze NPS has been developed and validated similar as we reported before in O'Rourke and Subedi, 2020.[20] Briefly, 50 mL of acidified (HCl, pH~2) raw wastewater was centrifuged at 10,000 rpm for 5 minutes and filtered under vacuum using 0.45 µm nylon filter paper to separate suspended particulate matter. The wastewater samples were spiked with internal standards, mixed well, and extracted using Oasis® MCX 6 cc solid-phase extraction cartridges. 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 approximately five minutes, 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 vial and the final volume was adjusted to ~500 uL with methanol. One microliter of all prepared samples was subjected to the instrumental analysis. Instrumental analysis was the same as described above for the stimulants.

Comparative Analysis

The analytic data required for the comparative analyses were acquired through data use agreements (DUA), established partnerships through this grant, and/or publicly available data sources. A DUA was executed with the KY CHFS to access KY All Schedule Prescription Electronic Reporting (KASPER) or PDMP stimulant prescribing data. This data was also used for a multistate, amphetamine prescribing trends poster and interactive website (see https://public.tableau.com/app/profile/ipop/viz/WAViz/Home) presented at the Rx and Illicit Drug Summit (2024) where the National Institute of Justice (NIJ) was acknowledged for funding the KY team's acquisition of data. An example data analysis from that poster was that KY's amphetamine Rx rate increased by 21% from 3.8 Rx per 100 in 2019 Q4 to 4.6 Rx per 100 in 2022 Q4. **Understanding the background levels of prescribed substances, such as amphetamines, that are also metabolites of illicit substances (e.g., methamphetamine metabolizes to amphetamine) is critical for moving the field forward.**

Table 2 details all data generated and collected for this project. Using the KY stimulant prescribing data, trends in stimulant use were examined at the zip code level allowing for a high-resolution view of prescribing "hot spot" areas of the state. The comparative analysis also involved contrasting wastewater results from roadway facilities with those from WWTFs to assess variability in drug profiles between roadway and community use. Unfortunately, we could not recruit WWTFs operating in the counties where the roadway facilities were located as

Table 2. WeTEST datasets submitted to the NIJ repository.

Description	Source
Calculated Mass Loads by Location and Stimulant Drug Type	UK/MSU
KY Motor Vehicle Crash Data, by Large Truck Involvement, 2019-2021	National Highway Traffic Safety Administration (NHTSA) Motor Vehicle Crash Data
Quarterly Stimulant Rates by County, 2019-2022	KY All Schedule Prescription Electronic Reporting (KASPER)
KY Hospital Utilization Data: Overdose-Related Emergency Department and Inpatient Visits, Fatal Overdoses, 2018-2022	KY Injury Prevention & Research Center (KIPRC)
Drug-Related Offenses, 2020-2022	National Incident-Based Reporting System (NIBRS)

originally planned. As such, wastewater results were effectively compared with other population-based drug use indicators to further understand drug use patterns and discrepancies.

The study protocol was submitted to and approved by the UK Institutional Review Board (IRB# 59087), ensuring compliance with the Department of Justice Human Subjects Protection regulations (28 CFR Part 46) and qualifying for exemption under Section 46.101(b) Category 4.

Expected Applicability of the Research

The research outcomes of this study serve as a valuable resource for authorities to map "hot spots" of drug flow, assess trends over time or seasons, prompt rapid intervention, identify new drugs of use, and correlate the drug use activities with multiple public health and safety indicators. As results and awareness of **WeTEST** spread, Dr. Delcher responded to multiple inquiries from interested federal agencies including the U.S. Drug Enforcement Administration, U.S. Homeland Security, and the Office of the National Drug Control Policy.

PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

This research established a unique collaboration between UK, MSU, the KY Transportation Cabinet, the KY CHFS, and other agencies. Preliminary meetings were held with leadership in the Appalachian HIDTA office at the beginning of the project, but collaboration efforts were interrupted by the COVID-19 emergency period in early 2020. As a result of this project, Dr. Delcher joined a new wastewater team focused on WWTFs in multiple cities that formed during the project period led by the University of Florida and now the University of South Carolina (funded by the National Institute on Drug Abuse). Dr. Delcher regularly provides insight into WBE and **WeTEST** results/experience are frequently shared to help contextualize findings in that project.

CHANGES IN APPROACH

This research involved extensive wastewater sampling over the course of multiple years. Given constraints on personnel and sampling equipment as **WeTEST** proceeded, we reduced the number of rest areas originally planned for the study. We developed a plan to keep seven out of the original eight sampling sites (see **Figure 4**) and, with approval from NIJ, we removed one planned truck weigh station. Our recruitment of WWTFs was limited to the four locations in the eastern part of the state that were already participating in a different COVID-19 related sampling project. That recruitment and sampling was supported by a pilot grant from the University of KY's Substance Use Priority Research Area (SUPRA) and National Institutes of Health (NIH) grant # 1U01DA053903-01 and P30 ES026529 who are gratefully acknowledged.

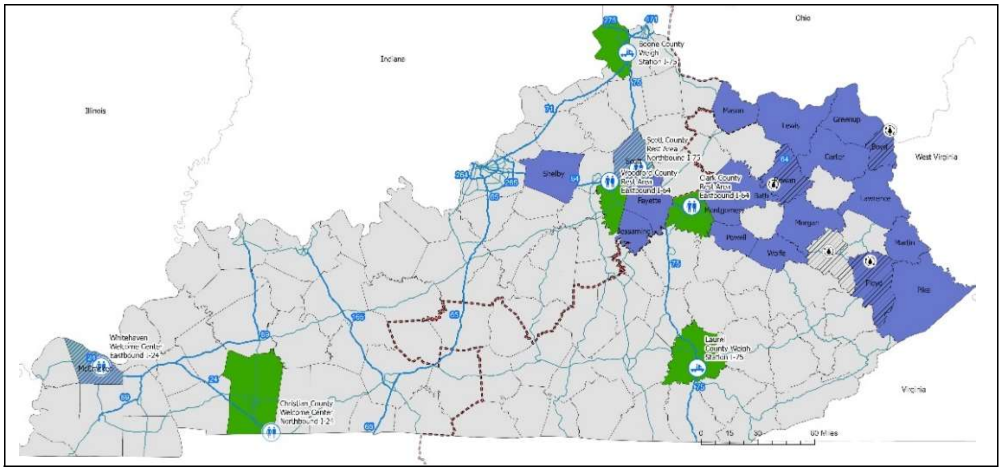

Figure 4. WeTEST sampling sites. Final locations of rest areas (two welcome centers, three rest areas), two truck weigh stations and four wastewater treatment facilities.

OUTCOMES

Results and Findings

During the project, we published two peer-reviewed manuscripts, delivered multiple poster presentations, and participated in various speaking engagements that addressed important and extensive aspects of our research. This final report will focus on additional insights, hypotheses, and/or preliminary analytical perspectives of our research that have not been published elsewhere. Given the large number of stimulant substances that we examined (see **Table 3**), the report focuses on just a few to illustrate impactful findings. Complete data files have been uploaded to NIJ's repository which should allow for replication and verification of findings by external researchers in the future. The following findings are organized by research question.

Table 3. Target stimulants examined by **WeTEST** (metabolites are italicized).

Illicit Stimulants	**NPS-Stimulants**
Cocaine	1-(3-Chlorophenylpiperazine)
Benzoylecgonine	para-fluorofentanyl
Cocaethylene	MAB-CHMINACA
	3,4-Methylenedioxypyrovalerone
Methamphetamine	4-Methyl Pentedrone
MDMA	2-Methyl-4'(methylthio)-2-Morpholinopropiophenone
MDA	Methcathinone
	Mitragynine
Prescribed Stimulants	Metonitazene
Amphetamine	
Methylphenidate	p-Hydroxymethamphetamine
Phentermine	

Research Question 1. What are the stimulant drug types detected in the general driving population along three major interstate highways (I-24, I-64, and I-75) in KY?

There are two perspectives on determining when drugs were detected in samples. The first is a strictly "qualitative" indication of a drug's presence in the sample (i.e., yes/no or positive/negative) based on the detection parameters for the analytical instrumentation. The second is an indication of a calculatable "mass load" (e.g., drug consumption rate) which is the ideal endpoint outcome for the project because it provides us with the estimated amount of drugs consumed by the population at the site. Both perspectives are frequently reported in the literature. **Figure 5** shows select **WeTEST** stimulant data from these two perspectives. For example, 95% of Christian County's 116 roadway samples were positive for amphetamines and 70.7% of samples yielded an acceptable mass load value. The reasons why a mass load fails to calculate can vary from features of the sample itself to a lack of ancillary data needed to complete the mass load calculation such as human excretion rates, water flows at the facilities, and/or stable population estimates from the facilities. In the case of the latter, we have complete records/notes by day and site that indicate when water flow estimates (e.g., water meters were broken and unreadable) or population estimates (e.g., the door-mounted people counters stopped working) were unavailable. Our **WeTEST** online dashboard also provides ancillary data on water flow and populations passing through roadway facilities collected by month and site. Additional rapid observations from **Figure 5** include: the drug with the most mass loads calculated was methamphetamine with 518 of 637 (81%) and the least was cocaethylene with only 150 of 637 total samples (23.5%). Cocaethylene is present only when cocaine is consumed with alcohol. **One general conclusion is that samples from the two trucking weigh stations had fewer positive samples for both cocaine and cocaethylene**.[21] From a public health and safety perspective, this is positive finding given the impact that crashing a large truck could have. At the Boone County Truck Haven, 94.2% of samples (65 of 69 samples) had a mass load calculation available and the Clark County Rest Area had 89.3% (92 of 103 samples) with a mass load calculation. Novel stimulant substances were also detected and presented in the next section.

Location	Name	Total Samples	% Positive Detection					
			Amphetamine	Benzoylecgonine	Cocaethylene	Cocaine	Methamphetamine	Methylphenidate
CC	Christian County (RA)	116	95	91	22	77	95	60
CR	Boone County Truck Haven	66	100	95	0	68	100	41
GT	Scott County (RA)	74	100	100	15	100	100	81
LC	Laurel County Truck Haven	88	90	86	9	67	90	45
WC	Clark County (RA)	99	100	100	22	100	100	46
WF	Woodford County (RA)	63	100	100	35	100	100	79
WH	McCraken County (RA, Whitehaven)	116	100	92	52	86	100	34
Total		**622**	**97.9**	**94.9**	**22.1**	**85.4**	**97.9**	**55.1**

			Amphetamine			Benzoylecgonine			Cocaethylene			Cocaine			Methamphetamine		Methylphenidate		
Location	Name	Total Samples	N	Y	% Y	N	Y	% Y	N	Y	% Y	N	Y	% Y	Y	%Y	N	Y	% Y
CC	Christian County (RA)	116		82	70.7		82	70.7	42	40	34.5		82	70.7	82	70.7	44	38	32.8
CR	Boone County Truck Haven	69		65	94.2	3	62	89.9	65		0.0	20	45	65.2	65	94.2	38	27	39.1
GT	Scott County (RA)	78		66	84.6		66	84.6	56	10	12.8		66	84.6	66	84.6	12	54	69.2
LC	Laurel County Truck Haven	91	12	60	65.9	13	59	64.8	50	22	24.2	10	62	68.1	72	79.1	26	46	50.5
WC	Clark County (RA)	103		92	89.3		92	89.3	70	22	21.4		92	89.3	92	89.3	49	43	41.7
WF	Woodford County (RA)	67		58	86.6		58	86.6	38	20	29.9		58	86.6	58	86.6	11	47	70.1
WH	McCraken County (RA, Whitehaver	113		83	73.5	4	79	69.9	47	36	31.9	10	73	64.6	83	73.5	59	24	21.2
Total		**637**	**12**	**506**	**79.4**	**20**	**498**	**78.2**	**368**	**150**	**23.5**	**40**	**478**	**75.0**	**518**	**81.3**	**239**	**279**	**43.8**

Figure 5. Samples positive for select stimulants (top) and samples where mass loads could be calculated (bottom).

Research Question 2. Are there any novel stimulant drug types detected during the project study period?

Our findings confirm the presence of multiple new stimulant compounds. **This discovery is significant, as it reflects the continuously changing landscape of stimulant use and emphasizes the need for ongoing monitoring and research.** The identification of these novel substances not only enhances our understanding of drug trends but also highlights potential new implications for public health and safety. Given the complexity of the laboratory work involved in creating new standards and other sensitivity issues with the instrumentation to accomplish this objective, this data has not been fully analyzed by substance, site, and time by our team. However, we have submitted the mass load results to NIJ's repository for future analysis.

Research Question 3. Do stimulant drug types align with documented stimulant drug problems in communities where the rest areas are located?

Fatal stimulant-involved overdoses. For this subanalysis, we selected and assessed the annualized five-year rates of fatal overdoses involving psychostimulants across the seven counties in our study: Boone, Laurel, Scott, Christian, Clark, Woodford, and McCracken, disaggregated according to the location of the two major facility types (rest areas (RA) and weigh stations (WS)) in KY from 2018 to 2022. The comparative dataset utilized for this study was obtained from the KY Injury Prevention and Research Center (KIPRC).[22] Population estimates were obtained from the U.S. Department of Commerce, Bureau of the Census, Population Estimates Program and 2020 Decennial Census.[23]

For data originally reported to us as fewer than ten fatalities (<10), we imputed the missing values by replacing them with random values[24] between one and nine. We then calculated five-year rates of fatal overdose per 100,000 population. Rates were obtained by taking the sum of the estimated overdose counts over five years (2018-2022) and normalizing it against a five-year population estimate (derived from the 2023 population estimate multiplied by five) as shown below:

$$Five - Year\ Rate\ per\ 100{,}000 = \left(\frac{Total\ Overdose\ Counts}{Population\ Estimate\ x\ 5}\right) x\ 100{,}000$$

We calculated rates for fatal overdoses involving cocaine, stimulants other than cocaine, and all stimulants. Fatal overdoses in KY are not disaggregated to methamphetamine or other stimulant types.

Descriptive mass load correlations. From a conceptual perspective, it is difficult to directly compare cocaine-involved overdoses in the county's general population where the truck weigh stations are located as it is highly unlikely that cocaine use in the trucking population is correlated with use in the county population given the transient nature of the trucks and drivers passing through the weigh stations. There may be higher correlation in the rest areas especially if a large proportion of the county's population uses the rest area. However, we could not find any information on in-county versus out-of-county rest area use ratios. It is not entirely clear (see scale of mass load measurement for cocaine) if Woodford County has a higher rate of cocaine overdoses *and* mass loads in its rest area compared to the other rest areas with measurements available (see **Figures 6 and 7**). Mass loads with confidence intervals

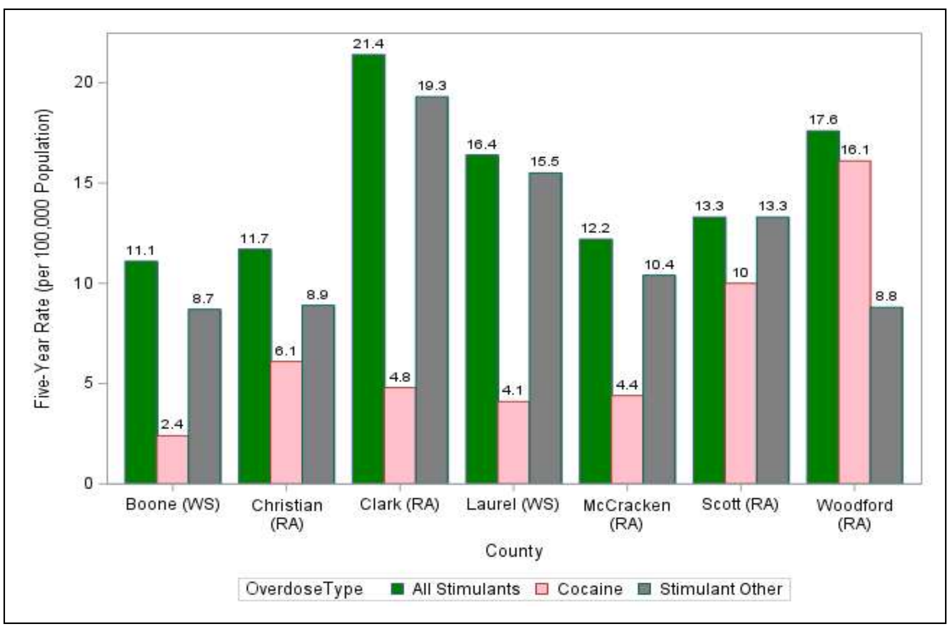

Figure 6. Annualized Fatal Overdose Rates by County location of facilities in the **WeTEST** study and Drug Type.

are provided in full on our dashboard.

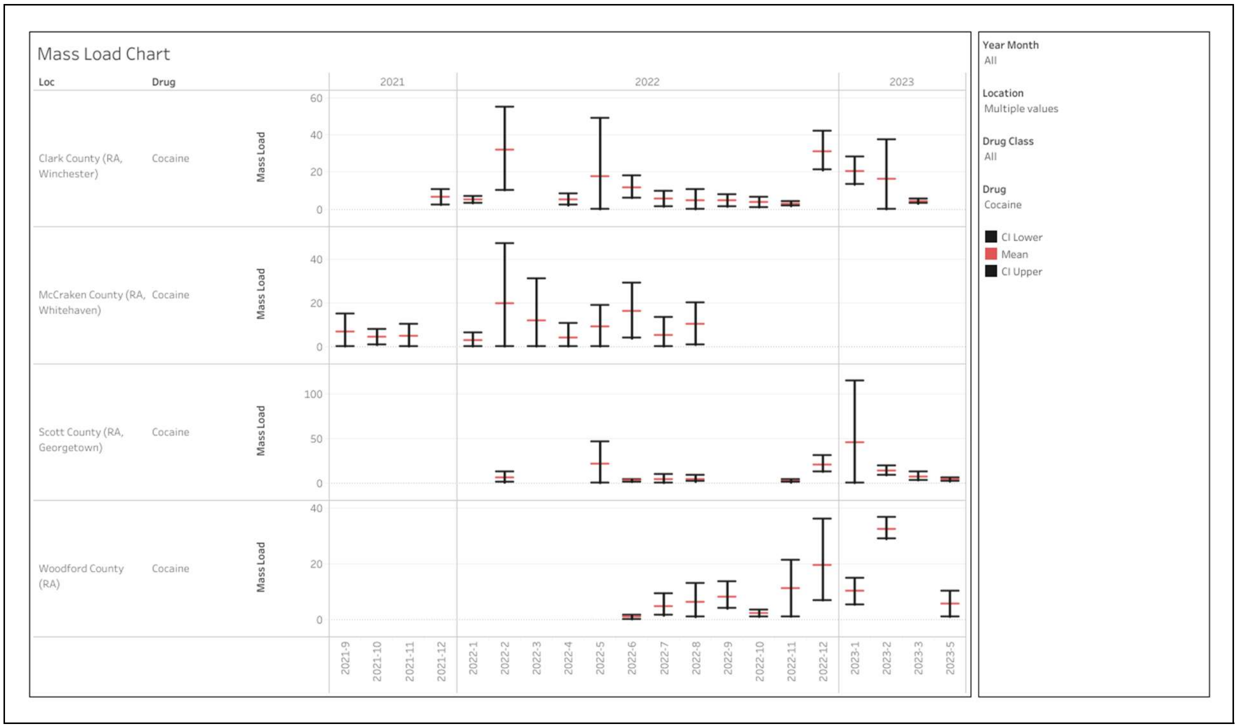

Figure 7. Mass loads with error bars by select roadway facility for cocaine. The cocaine metabolite benzoylecgonine is available on the website but not shown here. This is a screen shot from our interactive data query system.

Research Question 4. Does stimulant drug prevalence in rest areas correlate with the other reported public health and safety indicators such as road accidents, associated drug crime and/or controlled substance prescribing patterns?

Road accidents. In the U.S., the most recent national roadside survey of drivers (2013-2014 indicated that more than 15% of drivers tested positive for at least one illicit drug.[25] Among fatally injured motor vehicle drivers in KY (excluding alcohol and antidepressants), cannabinoids (15.8%), opioids (e.g., hydrocodone) (14.9%), and stimulants (e.g., amphetamine) (5.80%) were the most frequently identified drugs in 2010-2014.[26] According to the Federal Motor Carrier Safety Administration, approximately 3% of large truck drivers involved in fatal crashes were positive for a stimulant (2018-2020).[27] For this subanalysis, we obtained data from the National Highway Traffic Safety Administration (NHTSA) Motor Vehicle Crash Data on fatal accidents involving large trucks.[28] **Our findings indicate that the percentage of fatal crashes involving large trucks is generally higher in Boone County (~30%) as compared to Laurel County (<20%), which appears to correlate with higher mass loads of methamphetamine in Boone County (see Figure 8)**. However, it is important to note that the sample size for the crash data analysis was extremely small and we do not know if these truck-involved fatalities involved drug use. This is a trend worth exploring but a more extensive analysis is needed to draw definitive conclusions.

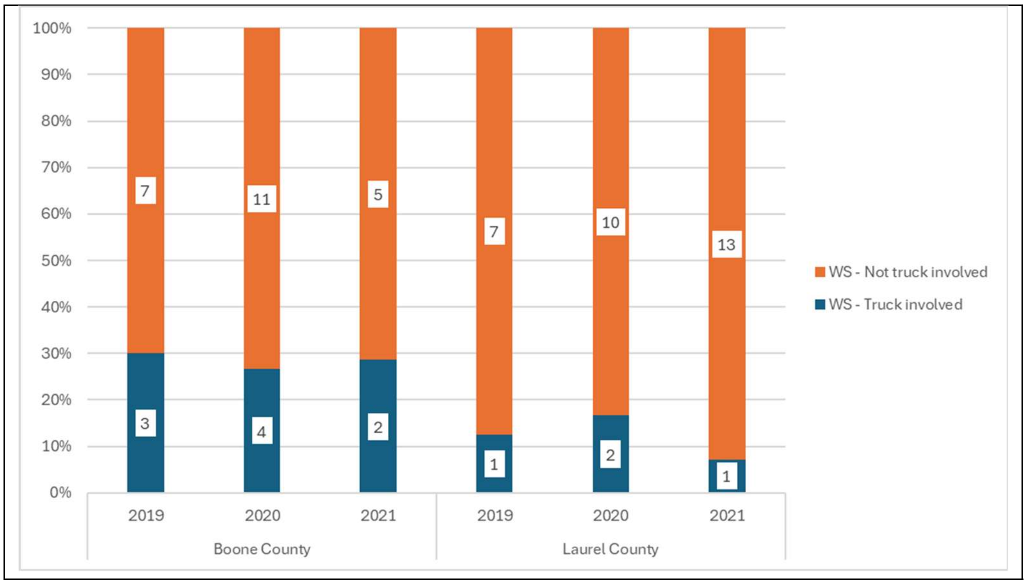

Figure 8. The percentage and number of fatal crashes involving large trucks in Boone and Laurel County (truck weigh station locations).

Drug crime. For this subanalysis, we used data from the National Incident-Based Reporting System (NIBRS).[29] Among the counties in our study, we found that drug related offenses were highest in Boone, Laurel, and McCraken Counties (see **Figure 9**). Laurel County is located in the KY Appalachian High Intensity Drug Trafficking Area. These results may indicate high law enforcement activity in these counties.

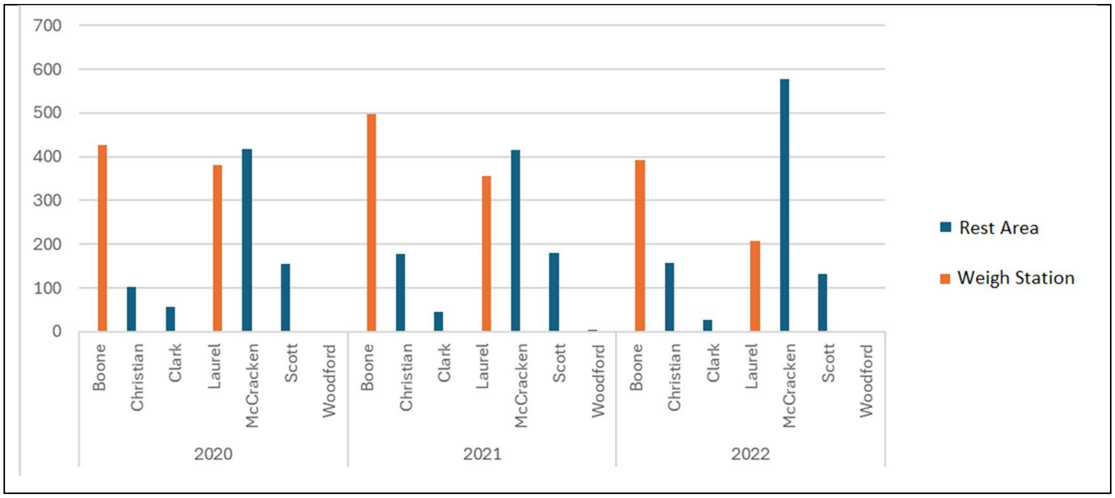

Figure 9. Drug related offenses (no.) by select county in **WeTEST**.

Prescribing data. One of the most exciting aspects of our project from a public health and public safety integration perspective was the ability to obtain stimulant prescribing data from KASPER. As a field, WBE suffers from very few studies that correlate multiple drug surveillance outcomes with WBE outcomes. As previously discussed, the involvement of psychostimulants with abuse potential in fatal overdoses has increased. According to the U.S. Centers for Disease Control and Prevention (CDC) there were 57,597 psychostimulant involved deaths in 2022.[30] In a national study of commercial claims, approximately 40% of youth with an overdose involving amphetamine or methylphenidate had a stimulant prescription dispensed in the prior six months.[31] These co-occurring and increasing trends, along with studies showing the intersection of prescribed and illicitly manufactured stimulants, have renewed concerns over the abuse, diversion, and overdose potential for legal, prescription stimulants.[32]

To identify prescription stimulants in the KASPER files, we linked National Drug Codes (NDC) available in the records to Medi-Span® with the therapeutic class group product identifier (GPI) 61 and grouped NDC by active stimulant ingredient per standard methodology.[33] For this subanalysis, we examined two major active ingredients, amphetamines (56.2%) and methylphenidate (24.9%), which accounted for 81% of stimulants dispensed in this period (data not shown). Phentermine accounted for 16% of all stimulants and was excluded from this analysis. Each patient's residential ZIP code was linked to a single ZIP Code Tabulation Area (ZCTA) available from the U.S. Census Bureau.[34] Eleven (1.2%) ZCTAs could not be uniquely assigned so we assigned these to the ZCTA that contained the largest proportion of the ZIP code's population.

To examine county sociodemographic context, we downloaded five measures of the 2018 Social Vulnerability Index (SVI) from the CDC for Toxic Substances and Disease Registry: 1) Socioeconomic Status, 2) Household Characteristics, 3) Racial and Ethnic Minority Status, 4) Housing Type and Transportation, and the Summary Ranking.[35] The SVI is designed to measure community disaster preparedness and has been used to examine health outcomes including the distribution of prescription medication for opioid use disorder and overdoses from psychostimulants.[36,37] The SVI assigns each tract a score based on percentile rank (scored zero to one, with one representing the highest vulnerability in any given category).

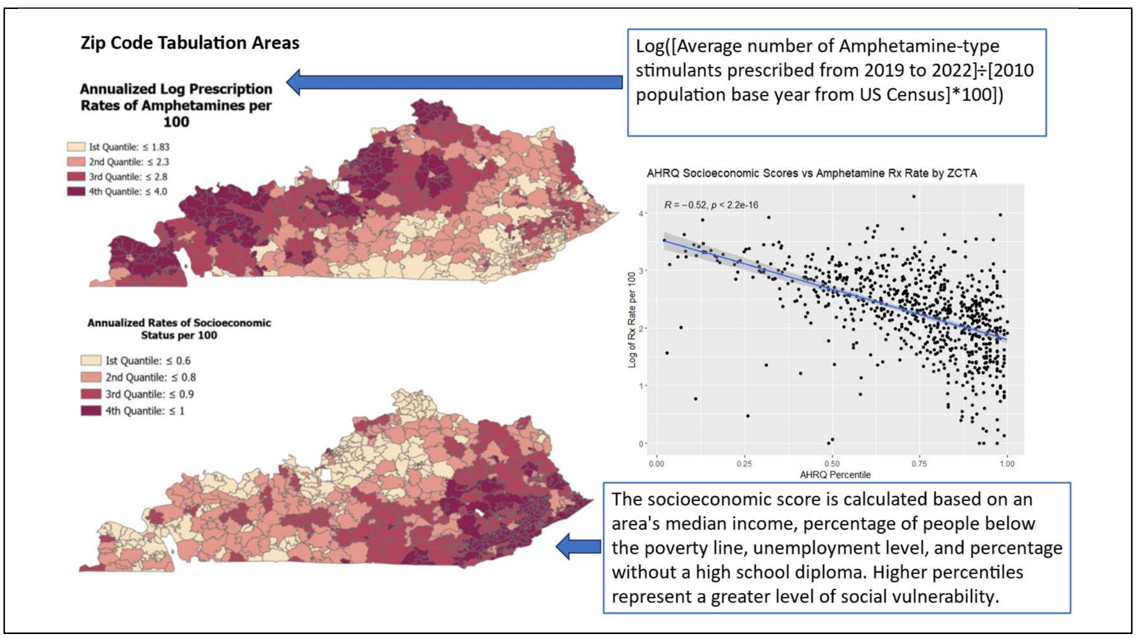

Figure 10. Amphetamine prescribing and socioeconomic scores by zip code in KY, 2019-2022. Data for this analysis were obtained by agreement with the KY All Schedule Prescription Electronic Reporting (KASPER) system.

We found that amphetamine prescribing was generally higher in the Western part of the state. It is well-known that socioeconomic status is lower in the Eastern part of the state and the socioeconomic score gradients reflect that (see **Figure 10**). Breaking down our results by eastern and western rest areas, there is no obvious difference in the amphetamine mass loads but testing this hypothesis may require more sensitive statistical analysis. For additional information on how we used stimulant prescribing data see **Research Question 7**.

Research Question 5. What are the stimulant drug types used by truck drivers along interstate trucking facilities?

Given the staggered nature of our sampling strategy, it is important to understand the consistency in sampling days between sites and which sites have mass load data available over comparable periods to answer this question. For example, there are seven months of overlap sampling for the two weigh stations in Boone County and Laurel County. **Table 4** helps us see that amphetamine, methamphetamine, and cocaine have mass load calculations available for most months in both sites. Since methamphetamine mass loads were available at both weigh stations for this 7-month period, we focused the question on this drug.

Table 4. Mass Load Calculations for Boone and Laurel County, July 2022 – May2023.

	Amphetamine		Methamphetamine		Cocaine		Methylphenidate		Benzoylecgonine		Cocaethylene	
Month/Year	Boone	Laurel	Boone	Laurel	Boone	Laurel	Boone	Laurel	Boone	Laurel	Boone	Laurel
Jul-22	x	x	x	x		x		x	x	x		
Sep-22	x	x	x	x	x	x		x	x	x		
Oct-22	x	x	x	x	x	x			x	x		
Nov-22	x	x	x	x		x		x	x	x		x
Jan-23	x	x	x	x	x	x		x	x	x		x
Mar-23	x		x	x	x	x	x	x	x			
May-23	x	x	x	x	x	x	x	x	x			

Methamphetamine mass loads were consistently higher in Boone County compared to Laurel County except for September 2022 (see **Figure 11**). In wastewater epidemiology, unusually large mass load values can occur due to “dumping event” or could reflect errors in measurement so we cannot be certain that these spikes are accurate representations of drug use. We previously reported a two-month mean consumption rate of 56.8 (95%CI (0.70−113)) mg/day/1000 people in Laurel County which gives us an idea of baseline ranges. The September 2022 value is clearly outside of the expected range for both locations and the January 2023 value appears to be an outlier for Boone County as well.

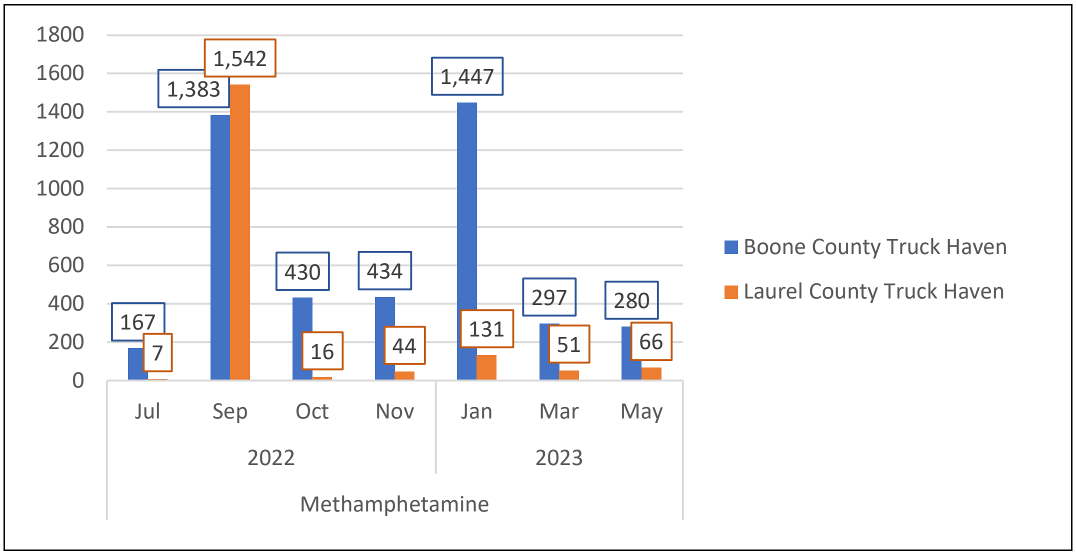

Figure 11. Methamphetamine consumption rates for Boone and Laurel County, July 2022 – May 2023.

Research Question 6. Are stimulant drug types different between truck drivers and the general population?

We addressed this question extensively in our first peer-reviewed publication. We refer the reader to that paper for full details.[38] **Figure 12** presents stimulant findings from that manuscript which shows lower methamphetamine mass loads from the Laurel County truck haven relative to the Christian County Welcome Center/rest area. The other stimulants are shown within overlapping uncertainty intervals. **Our online dashboard allows users to see all the drugs examined in the WeTEST project.**

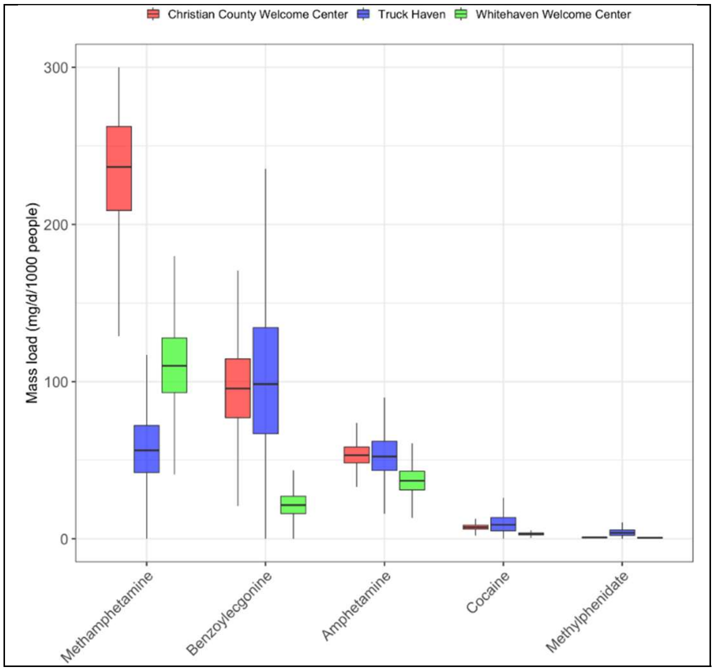

Figure 12. Figure from Gray et. al, 2022.

Research Question 7. Do stimulant drug types differ based on traffic entering/leaving the state along the TN border? Are stimulant drug types different based on travel direction?

In general, we found that the mass load of methamphetamine drugs from I-24 West (Christian County, TN border) were higher in 2021 but then returned to similar levels in 2022 compared to those from I-24 East (McCraken County, IL border). We discuss the comparative findings in more detail in **Research Question 9** from a temporal perspective, but we hypothesize that our wastewater observations are partially reflective of the drug epidemiology in this region. According to the CDC, TN ranked second in drug overdose deaths (56.6 per 100,000) whereas Illinois (IL) ranked 30th (29 per 100,000) (see **Figure 13**). Additionally, TN ranks 2nd for methamphetamine deaths (23.1 per 100,000) only behind KY (24.9 per 100,000).

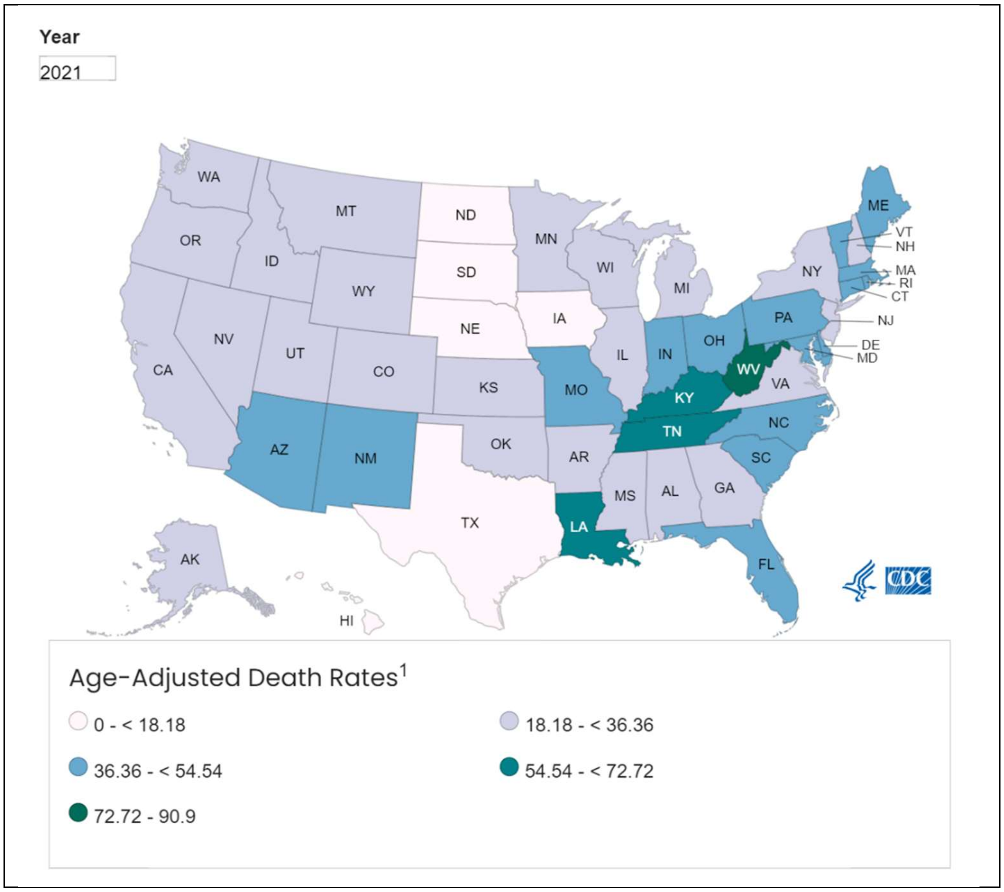

Figure 13. Age-adjusted death rates from drugs by state, 2021.
Image from the U.S. Centers for Disease Control and Prevention.

Research Question 8. Do interstate stimulant drug types correlate with drug detected from a nearby municipal water plants?

One of the subobjectives of **WeTEST** was to examine stimulant mass loads in WWTFs located in proximity to our roadway facilities. Logistically, we could not recruit nearby facilities; however, we took advantage of an opportunity to include four WWTFs in Eastern KY and compare their results with stimulant dispensing data obtained from KASPER for **WeTEST**. To compare mass loads to prescribing rates, we calculated an annualized four-year dispensing rate (2019-2022) by summing the annual number of total stimulant dispensations by active ingredient and dividing by the population living in the ZIP code (2010) multiplied by four. The zip code service areas were determined using ArcGIS to spatially identify the location of sewer pipes extending from the WWTF obtained from the KY Infrastructure Authority.[39] Highlights from the subanalysis include:

- Consistency in the amphetamine and methylphenidate mass loads from wastewater trends relative to four-year dispensing rates from retail pharmacies in the zip code service areas of the WWTFs. For example, samples from WWTF "A" had the highest mass loads and dispensing rates of both types of stimulants compared to all other locations (see **Figure 14**).
- Amphetamine and methylphenidate mass loads were much higher in samples from WWTF "A" than the other locations.
- Amphetamine consumption was consistently higher than methylphenidate across all locations. This relationship was also true for dispensing rates.
- The differences in amphetamine and methylphenidate dispensing were not as stark as differences between the mass load values for these drugs in wastewater. In other words, the variability was higher with mass load values. It is worth noting that excretion rates for methylphenidate are much lower (1.5%) than amphetamine (33%) which may account for some the of the higher disparity between mass loads and prescribing for this drug (see Gray et al. 2022).

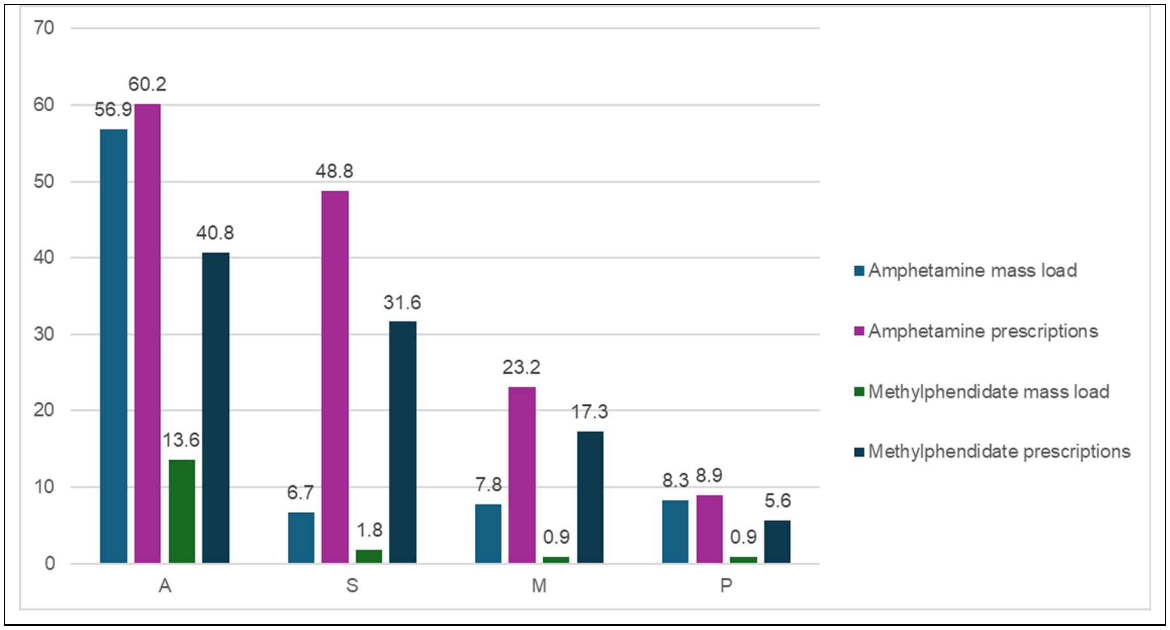

Figure 14. Amphetamine and methylphenidate mass loads and dispensing rates for four WWTFs in Eastern KY. Mass loads are mg/day/person and prescriptions are prescribing rates annualized for four years of data but have similar scales.

Research Question 9. Are there daily, monthly, and seasonal variations in stimulant drug types in the general population and/or truck drivers?

Holiday analysis. In the U.S., six of the ten highest rates of traffic casualties occur during federal holidays such as New Year's, the 4th of July, Labor Day, and Christmas.[40] **WeTEST** sampled several major holidays during the project period. In general, methamphetamine discharged on holidays was comparable to those on typical weekdays except for Labor Day, suggesting a steady discharge from highway commuters (see **Figure 15**). Conversely, cocaine and amphetamine discharged during Christmas, Memorial Day, the 4th of July, and Labor Day were higher than those on typical weekdays in their respective months. However, the average discharge of drugs during holiday periods (spanning three to five days) were not different from the average discharge during typical weeks (seven days).

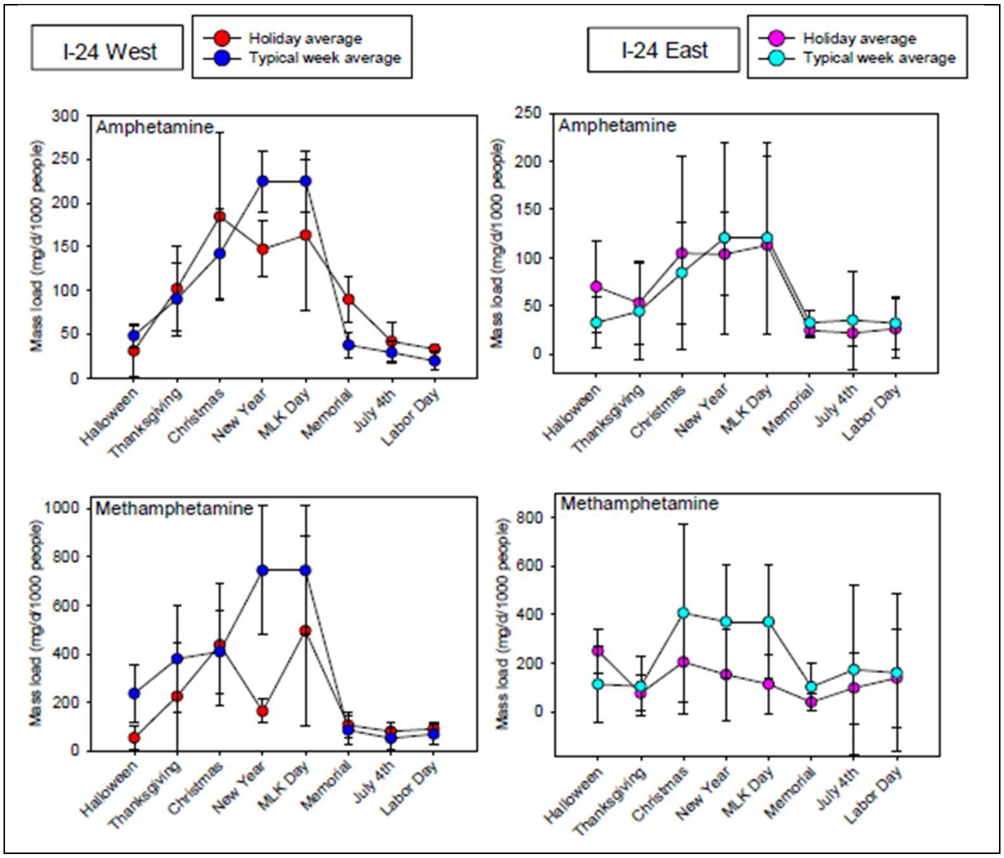

Figure 15. Mass loads measured during major holidays and typical weeks from two rest areas. Values represent averages across multiple years, based on the available samples at the time of analysis.

Temporal trends. The time trends in the Christian County I-24 West (TN border) and McCraken County I-24 East (IL border) locations were very consistent. From October 2021 to January 2022, mass loads were higher in Christian County followed by a large decline throughout the rest of 2022. Amphetamine and methamphetamine levels in both counties were observably lower in 2022 than 2021 as were cocaine and benzoylecgonine. **The decline from 2021 to 2022 is consistent with national and regional trends reported by the U.S. Drug Enforcement Administration (Figure 16) and may represent an important correlation between wastewater measurements and drug seizures.**[41]

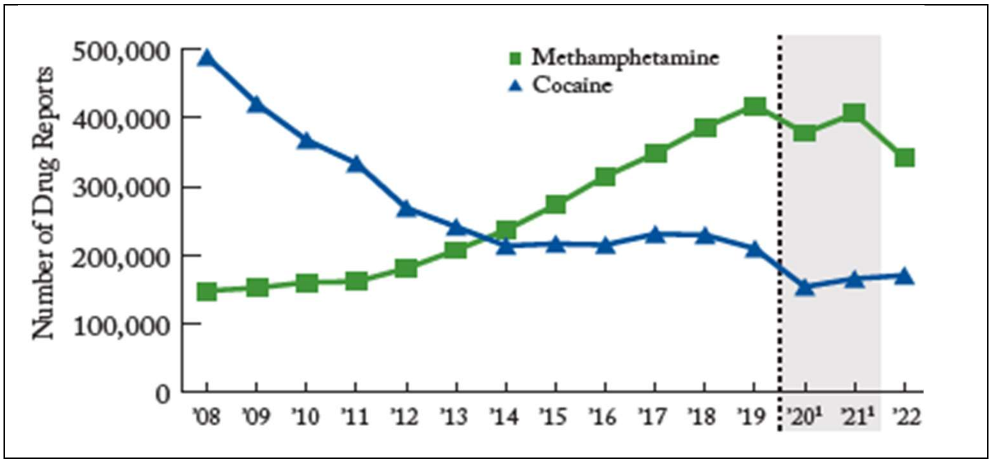

Figure 16. National trend estimates for methamphetamine and cocaine, January 2008–December 2022.

To examine in state detail, we downloaded state level data from the National Forensic Laboratory Information System (NFLIS) public data query system[42] to examine methamphetamine and cocaine seizures in KY and the states bordering those rest areas (TN, IL). **Regional trends for methamphetamine declines were consistent with national trends from 2021 to 2022 (Figure 17).**

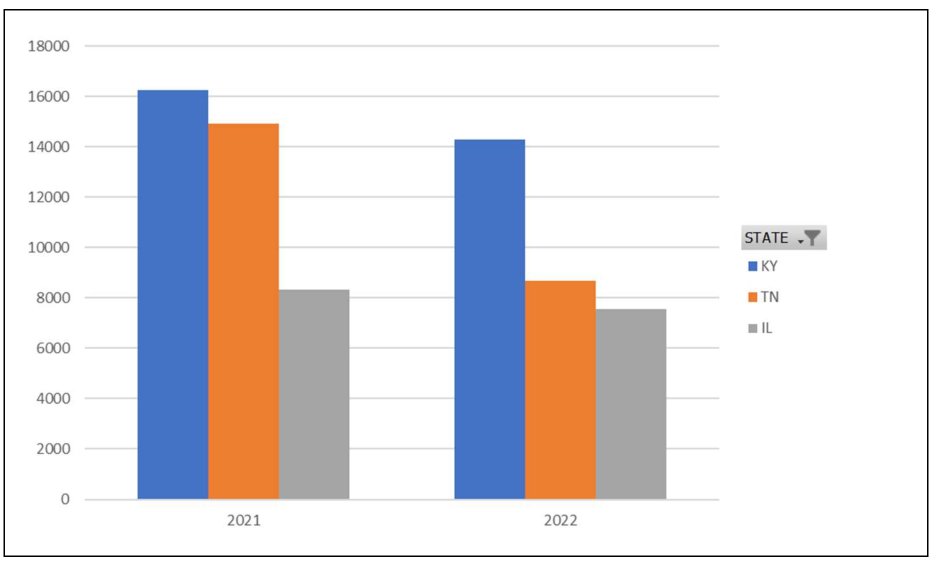

Figure 17. Methamphetamine positive drug seizures from NFLIS, 2021, 2022 for KY, TN, and IL (unadjusted).

The wastewater mass loads are higher in the rest area along the TN border (Christian County) compared to the IL border (McCraken County) which is a relationship reflected in the state

NFLIS report and consistent with regional epidemiology. For cocaine and its metabolite, benzoylecgonine, the relationship between the two rest areas changes abruptly between 2021 and 2022 (see **Figure 18**). Christian County is consistently higher in late 2021 but McCraken County mass loads exceed those of Christian County throughout the remainder of the sampling period. It is not clear if these abrupt changes in the magnitude and relationship between methamphetamine and cocaine are related to the same underlying mechanism (i.e., a possible large scale public health or safety intervention that we are unaware of). None of the other sites were consistently sampled over this time frame to understand the extent of this rapid change.

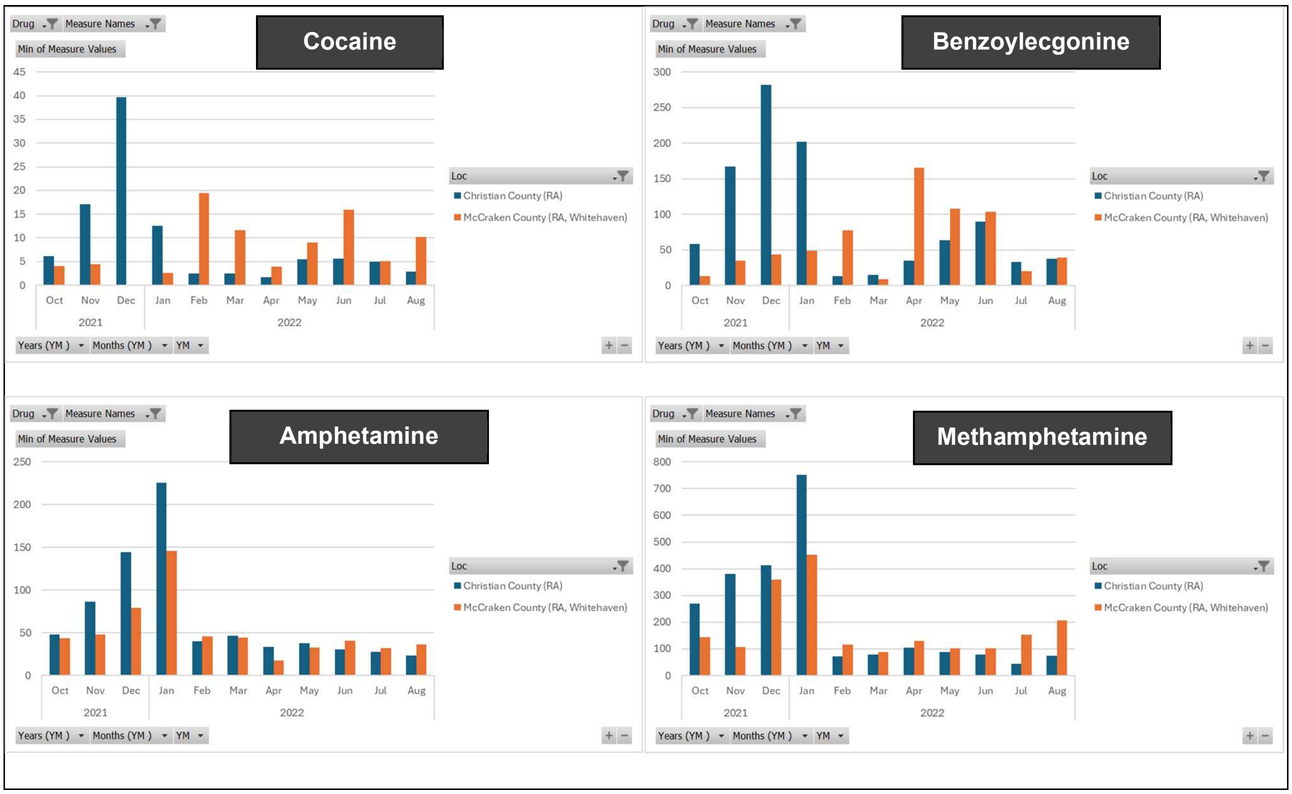

Figure 18. Cocaine, benzoylecgonine, amphetamine, and methamphetamine mass loads at two rest areas along the TN and IL borders with KY. Christian County (TN border) and McCraken County (IL border).

We examined the variability by site on an annual basis to discern additional trends and correlation to NFLIS. In general, mass loads for all four drugs appeared to increase from 2021 to 2023 with some inconsistencies (see **Figure 19**). **Increases in cocaine mass loads seem to be the most consistent with the increasing number of NFLIS drug seizures in KY**

(Figure 20). These results are not adjusted by population, nor do we have regular access to NFLIS data at the county level to understand the correlations to site level results. We attempted on multiple occasions to receive stimulant-involved laboratory submissions at the county level from the KY State Police during the project period but to no avail.

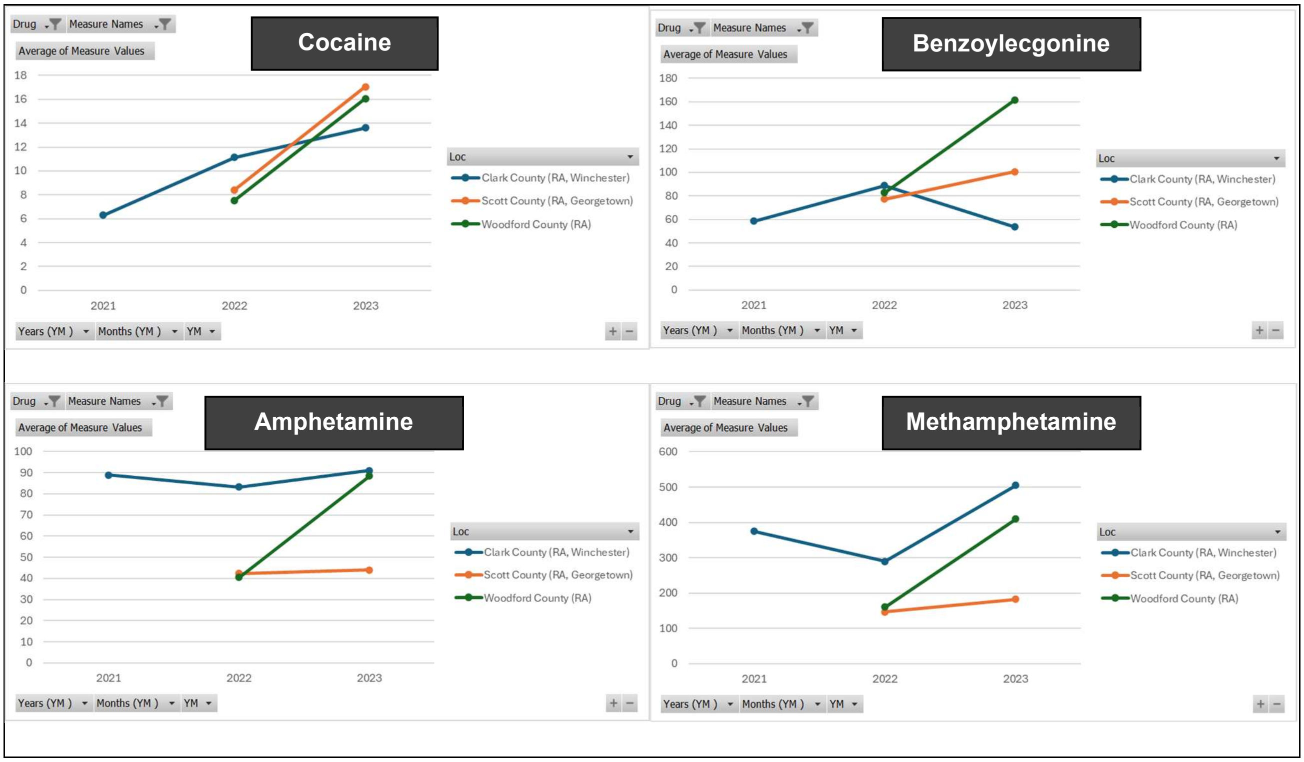

Figure 19. Annual, mean mass loads of four major stimulants at three KY rest areas.

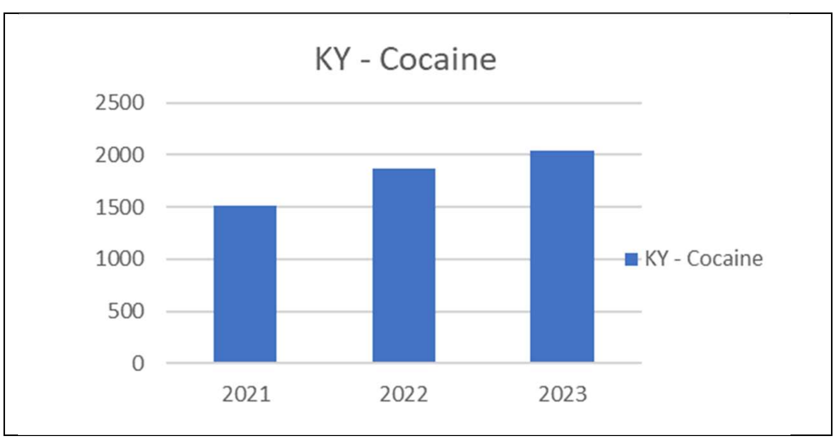

Figure 20. NFLIS submissions (no.) positive for cocaine in KY, 2021-2023.

Limitations

ARTIFACTS

List of Products

Multiple products were produced during the project period, including peer-reviewed publications, an interactive dashboard of project findings, and an NIJ recorded podcast. **Table 5** provides a detailed list of all products produced during the project period.

Table 5. WeTEST Products.

Publications
Delcher C, Quesinberry D, Torabi S, et al. Wastewater Surveillance for Xylazine in Kentucky. *AJPM Focus*. 2024;3(3):100203. doi:10.1016/j.focus.2024.100203
Gray KE, Delcher C, Shin E, et al. Drugs Discharged along Interstate Highway Restroom Facilities in Kentucky: Wastewater Analysis. *Environ Sci Technol Lett*. 2022;9(9):758-764. doi:10.1021/acs.estlett.2c00425
Websites
Shin, E. Wastewater Epidemiology To Examine Stimulant Trends (weTEST). Tableau Public. Published July 11, 2024. Updated July 19, 2024. https://public.tableau.com/app/profile/ipop/viz/WastewaterEpidemiologyToExamineStimulantTrendsweTESTupated012824/Front.
Murray State University. Collecting Wastewater Samples [Video]. YouTube. https://youtu.be/_MzO9Stg9yw?si=wXSeMnAF-mDcFGk3. Published August 5, 2021.
Podcast
Mullen L and Delcher C. Just Wastewater Drug Surveillance in Kentucky. [Just Science. Audio podcast episode]. National Institute of Justice's Forensic Technology Center of Excellence. 2024. https://forensiccoe.org/podcast-2024-cossup-ep3/

Datasets Generated

Table 2 lists the datasets generated and/or collected for this project.

Dissemination Activities

Research findings were disseminated at the local, state, and national levels to a range of audiences. These included fellow researchers and experts in the field, the CDC, the White House's internal wastewater group, NIJ leadership, and the NIJ Research conference. **Table 6** provides a detailed list of presentations delivered during the project period.

Table 6. WeTEST Presentations.

Oral Presentations
Delcher C and Subedi B. Wastewater Epidemiology To Examine Stimulant Trends. COEP Team Meeting; NYU Langone Health; 12 Dec 2023; Online.
Delcher C. Wastewater Surveillance Signals for Xylazine in Kentucky. Society of Environmental Toxicology and Chemistry 44th National Meeting; 16 Nov 2023; Louisville, KY.
Delcher C and Subedi B. Wastewater Epidemiology To Examine Stimulant Trends. NIJ Briefing; 13 Nov 2023; Online.
Delcher C and Subedi B. Wastewater Epidemiology To Examine Stimulant Trends. Drug Data Interagency Working Group (IWG); Office of National Drug Control Policy; May 2023; Online.
Delcher C. Wastewater Epidemiology To Examine Stimulant Trends. NIJ 2023 National Research Conference; May 2023; Arlington, VA.
Delcher C and Subedi B. Wastewater Epidemiology To Examine Stimulant Trends. Drug Data IWG Wastewater Sub Group; Office of National Drug Control Policy; May 2023; Online.
Delcher C. Wastewater-based epidemiology for drug detection: Epidemiological motivations. Chemistry Department Seminar; Murray State University; 16 October 2022; Murray, KY.
Poster Presentations
Jetson J, Shin E, Gautam P, Shannon J, Hammerslag L, Delcher C. Prescription Amphetamine Trends in Three US States. Poster presented at: Rx and Illicit Drug Summit; April 2024; Atlanta, GA.
Jones L. Drugs Discharged at Rest Areas and Truck Servicing Facilities during Federal Holidays in the United States. Poster presented at: Society of Environmental Toxicology and Chemistry 44th National Meeting; 16 Nov 2023; Louisville, KY.
Torabi et al. Wastewater Analysis to Monitor Substance Use Trends in Eastern Kentucky Communities. Poster presented at: Substance Use Research Event; Apr 2023; Lexington, KY.
Windhorst et al. Wastewater-based Epidemiological Determination of Drug Discharged at the Rest Areas During Federal Holidays in the U. S. Poster presented at: National American Chemical Society Meeting; Mar 2022; Chicago, IL.
Grey et al. Wastewater-based Estimation of Substances Discharged at the Rest Areas along the State Highways in Kentucky. Poster presented at: Poster-at-the-Capitol; Mar 2022; Frankfort, KY.

AKNOWLEDGEMENTS

We would like to express our gratitude to everyone who contributed to the completion of this project (see **Figure 21**). We extend our appreciation to our research team for their dedication

throughout the project, as highlighted in some of our project photos (**Figure 22**). A special thank you to the staff at Laurel County Truck Haven for their assistance with wastewater sampling. We would also like to thank the NIJ for their support, which made this research possible.

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Figure 21. WeTEST project partners, staff, and trained students.

Figure 22. WeTEST project photos showing samplers, various manholes, and staff at the Laurel County trucking weigh station collecting samples.

REFERENCES

1. National Institute on Drug Abuse. Drug Overdose Deaths: Facts and Figures | National Institute on Drug Abuse (NIDA). August 21, 2024. Accessed September 24, 2024. https://nida.nih.gov/research-topics/trends-statistics/overdose-death-rates

2. Kariisa M, Scholl L, Wilson N, Seth P, Hoots B. Drug Overdose Deaths Involving Cocaine and Psychostimulants with Abuse Potential — United States, 2003–2017. *MMWR Morb Mortal Wkly Rep*. 2019;68(17):388-395. doi:10.15585/mmwr.mm6817a3

3. Powell D. Educational Attainment and US Drug Overdose Deaths. *JAMA Health Forum*. 2023;4(10):e233274. doi:10.1001/jamahealthforum.2023.3274

4. Black JC, Bau GE, Iwanicki JL, Dart RC. Association of Medical Stimulants With Mortality in the US From 2010 to 2017. *JAMA Intern Med*. 2021;181(5):707. doi:10.1001/jamainternmed.2020.7850

5. O'Donnell J, Gladden RM, Mattson CL, Hunter CT, Davis NL. *Vital Signs:* Characteristics of Drug Overdose Deaths Involving Opioids and Stimulants — 24 States and the District of Columbia, January–June 2019. *MMWR Morb Mortal Wkly Rep*. 2020;69(35):1189-1197. doi:10.15585/mmwr.mm6935a1

6. Slavova S, Freeman PR, Rock P, et al. Changing Trends in Drug Overdose Mortality in Kentucky: An Examination of Race and Ethnicity, Age, and Contributing Drugs, 2016-2020. *Public Health Rep*. 2023;138(1):131-139. doi:10.1177/00333549221074390

7. Steel, M., Mirzaian, M., Daniels, L. (2023). Kentucky Resident Drug Overdose Deaths, 2018–2022: Annual Report, Updated April 2023. Kentucky Injury Prevention and Research Center. - Google Search. Accessed September 24, 2024.

8. Slavova S, Quesinberry D, Hargrove S, et al. Trends in Drug Overdose Mortality Rates in Kentucky, 2019-2020. *JAMA Netw Open*. 2021;4(7):e2116391. doi:10.1001/jamanetworkopen.2021.16391

9. Hogg-Graham R, Carman A, Mays GP, Zephyr PMD. Geographic Variation in the Structure of Kentucky's Population Health Systems: An Urban, Rural, and Appalachian Comparison. Published online 2020. doi:10.13023/JAH.0203.04

10. Team Kentucky. Justice and Public Safety Cabinet. (2023). 2022 Overdose Fatality Report. Accessed September 24, 2024.

11. Daughton CG. Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM). *Sci Total Environ*. 2018;619-620:748-764. doi:10.1016/j.scitotenv.2017.11.102

12. $95.7 Million Approved for Kentucky Tornado Recovery | FEMA.gov. September 8, 2022. Accessed September 26, 2024. https://www.fema.gov/press-release/20220908/957-million-approved-kentucky-tornado-recovery

13. Murray State University. Collecting Wastewater Samples. YouTube Video. August 5, 2021. https://youtu.be/_MzO9Stg9yw?si=wXSeMnAF-mDcFGk3

14. Foppe KS, Hammond-Weinberger DR, Subedi B. Estimation of the consumption of illicit drugs during special events in two communities in Western Kentucky, USA using sewage epidemiology. *Sci Total Environ*. 2018;633:249-256. doi:10.1016/j.scitotenv.2018.03.175

15. Skees AJ, Foppe KS, Loganathan B, Subedi B. Contamination profiles, mass loadings, and sewage epidemiology of neuropsychiatric and illicit drugs in wastewater and river waters from a community in the Midwestern United States. *Sci Total Environ*. 2018;631-632:1457-1464. doi:10.1016/j.scitotenv.2018.03.060

16. Croft TL, Huffines RA, Pathak M, Subedi B. Prevalence of illicit and prescribed neuropsychiatric drugs in three communities in Kentucky using wastewater-based epidemiology and Monte Carlo simulation for the estimation of associated uncertainties. *J Hazard Mater*. 2020;384:121306. doi:10.1016/j.jhazmat.2019.121306

17. Postigo C, Lopez De Alda MJ, Barceló D. Analysis of drugs of abuse and their human metabolites in water by LC-MS2: A non-intrusive tool for drug abuse estimation at the community level. *TrAC Trends Anal Chem*. 2008;27(11):1053-1069. doi:10.1016/j.trac.2008.10.002

18. Delcher C, Quesinberry D, Torabi S, et al. Wastewater Surveillance for Xylazine in Kentucky. *AJPM Focus*. 2024;3(3):100203. doi:10.1016/j.focus.2024.100203

19. Biden-Harris Administration Designates Fentanyl Combined with Xylazine as an Emerging Threat to the United States | ONDCP. The White House. April 12, 2023. Accessed September 29, 2024. https://www.whitehouse.gov/ondcp/briefing-room/2023/04/12/biden-harris-administration-designates-fentanyl-combined-with-xylazine-as-an-emerging-threat-to-the-united-states/

20. O'Rourke CE, Subedi B. Occurrence and Mass Loading of Synthetic Opioids, Synthetic Cathinones, and Synthetic Cannabinoids in Wastewater Treatment Plants in Four U.S. Communities. *Environ Sci Technol*. 2020;54(11):6661-6670. doi:10.1021/acs.est.0c00250

21. Centazzo N, Frederick BM, Jacox A, Cheng SY, Concheiro-Guisan M. Wastewater analysis for nicotine, cocaine, amphetamines, opioids and cannabis in New York City. *Forensic Sci Res*. 2019;4(2):152-167. doi:10.1080/20961790.2019.1609388

22. Kentucky Drug Overdose and Related Comorbidity County Profiles, 2018 to 2022 | KIPRC. Accessed September 24, 2024. https://kiprc.uky.edu/programs/overdose-data-action/county-profiles

23. 13. U.S. (2024). Department of Commerce, Bureau of the Census, Population Estimates Program and 2020 Decennial Census.

24. Donders ART, Van Der Heijden GJMG, Stijnen T, Moons KGM. Review: A gentle introduction to imputation of missing values. *J Clin Epidemiol*. 2006;59(10):1087-1091. doi:10.1016/j.jclinepi.2006.01.014

25. Kelley-Baker T, Berning A, Ramirez A, et al. 2014 National roadside study of alcohol and drug use by drivers: Drug results (Report No. DOT HS 812 411). National Highway Traffic Safety Administration. Published online 2013.

26. Bunn T, Singleton M, Chen IC. Use of multiple data sources to identify specific drugs and other factors associated with drug and alcohol screening of fatally injured motor vehicle drivers. *Accid Anal Prev*. 2019;122:287-294. doi:10.1016/j.aap.2018.10.012

27. Large Truck and Bus Crash Facts 2021 | FMCSA. Accessed September 29, 2024. https://www.fmcsa.dot.gov/safety/data-and-statistics/large-truck-and-bus-crash-facts-2021#A3

28. National Highway Traffic Safety Administration. Fatality Analysis Reporting System (FARS) Encyclopedia. NCSA Data Resource Website.

29. NIBRS. Federal Bureau of Investigation. Accessed September 29, 2024. https://www.fbi.gov/how-we-can-help-you/more-fbi-services-and-information/ucr/nibrs

30. Abuse NI on D. Drug Overdose Death Rates | National Institute on Drug Abuse (NIDA). May 14, 2024. Accessed August 7, 2024. https://nida.nih.gov/research-topics/trends-statistics/overdose-death-rates

31. Bushnell G, Samples H, Gerhard T, Calello DP, Olfson M. Benzodiazepine and Stimulant Prescriptions Before Overdose in Youth. *Pediatrics*. 2022;149(4):e2021055226. doi:10.1542/peds.2021-055226

32. Board AR. Trends in stimulant dispensing by age, sex, state of residence, and prescriber specialty — United States, 2014–2019. *Drug Alcohol Depend*. Published online 2020:7.

33. Kluwer W. Medi-Span Generic Product Identifier.https://www.wolterskluwer.com/en/solutions/medi-span/about/gpi. 2024.

34. US Census Bureau. ZIP Code Tabulation Areas (ZCTAs). The United States Census Bureau. Accessed January 28, 2021. https://www.census.gov/programs-surveys/geography/guidance/geo-areas/zctas.html

35. CDC/ATSDR's Social Vulnerability Index (SVI). April 28, 2021. Accessed December 9, 2021. https://www.atsdr.cdc.gov/placeandhealth/svi/index.html

36. Joudrey PJ, Kolak M, Lin Q, Paykin S, Anguiano V, Wang EA. Assessment of Community-Level Vulnerability and Access to Medications for Opioid Use Disorder. *JAMA Netw Open*. 2022;5(4):e227028. doi:10.1001/jamanetworkopen.2022.7028

37. Segel JE, Shearer RD, Jones AA, et al. Understanding Regional Patterns of Overdose Deaths Related to Opioids and Psychostimulants. *Subst Use Misuse*. 2024;59(4):558-566. doi:10.1080/10826084.2023.2287220

38. Gray KE, Delcher C, Shin E, et al. Drugs Discharged along Interstate Highway Restroom Facilities in Kentucky: Wastewater Analysis. *Environ Sci Technol Lett*. 2022;9(9):758-764. doi:10.1021/acs.estlett.2c00425

39. Kentucky Wastewater Mapping. Accessed September 29, 2024. https://kygeonet.ky.gov/kia/cw/index.html

40. National Center for Statistics and Analysis (NCSA) | NHTSA. Accessed September 15, 2024. https://www.nhtsa.gov/research-data/national-center-statistics-and-analysis-ncsa

41. U.S. Drug Enforcement Administration, Diversion Control Division. 2022 NFLIS-Drug Annual Report. Published online September 2023.

42. US Drug Enforcement Administration. National Forensic Laboratory Information System Data Query System. 2023. Accessed May 16, 2023. https://www.nflis.deadiversion.usdoj.gov/publicDQSinfo.xhtml?jfwid=RyIGZcO24QcyyS0QID4E9JtRpZ3CUj48Cd3QRYEN:0

43. Subedi B. Estimation of Community Usage of Drugs Utilizing Sewage Epidemiology. *Methods Mol Biol Clifton NJ*. 2018;1810:141-147. doi:10.1007/978-1-4939-8579-1_14