

Application of fast scanning triple quadrupole tandem liquid chromatography-mass spectrometry (LC-MS/MS) in population health assessment

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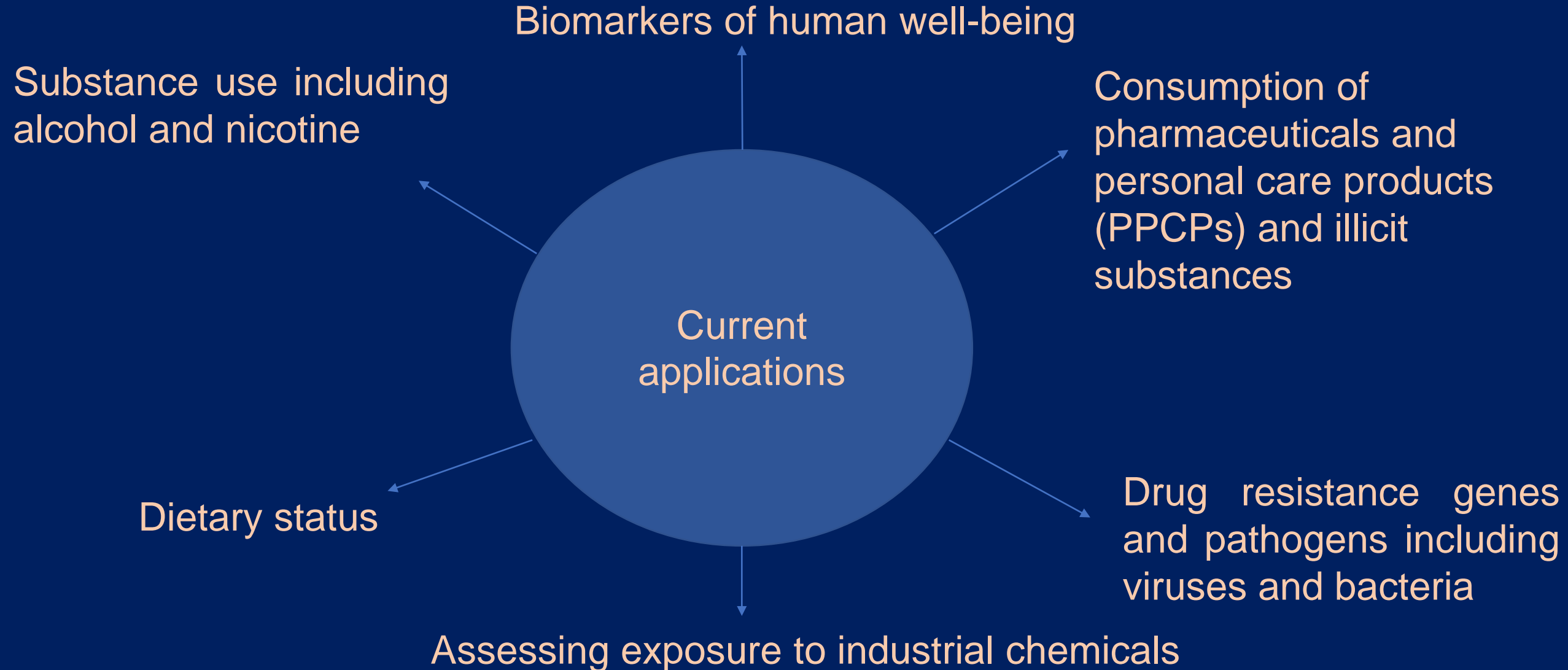
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Population health assessment

- **SARS-CoV-2 monitoring has emphasized the attractiveness of population health assessment using wastewater-based epidemiology (WBE)**
- **A rapid, non-invasive, and cost-effective public health surveillance tool at near-real time scale**
- **WBE is now being widely practiced and here we present some data from pre-COVID WBE adopters**

Monitoring public health status



Typical workflow of WBE study

Collection of community wastewater



Detection and quantification of signature chemicals

Flow data

Determination of mass load

Population estimate

Dose per capita calculation

Normalization for losses (Pharmacokinetics and in-sewer decay)

Estimate of consumption or exposure

Typical approach of sample processing and analysis workflow in our lab



Sample
Collection

Sample
Prep /
Internal Std.
Addition

Sample
Extraction

Elution

Extract
Blowdown
Reconstitution

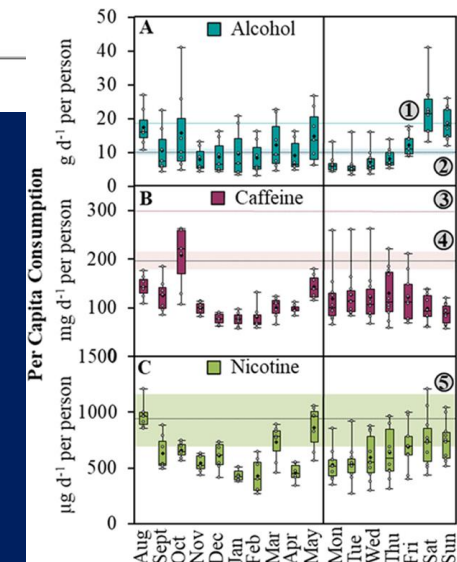
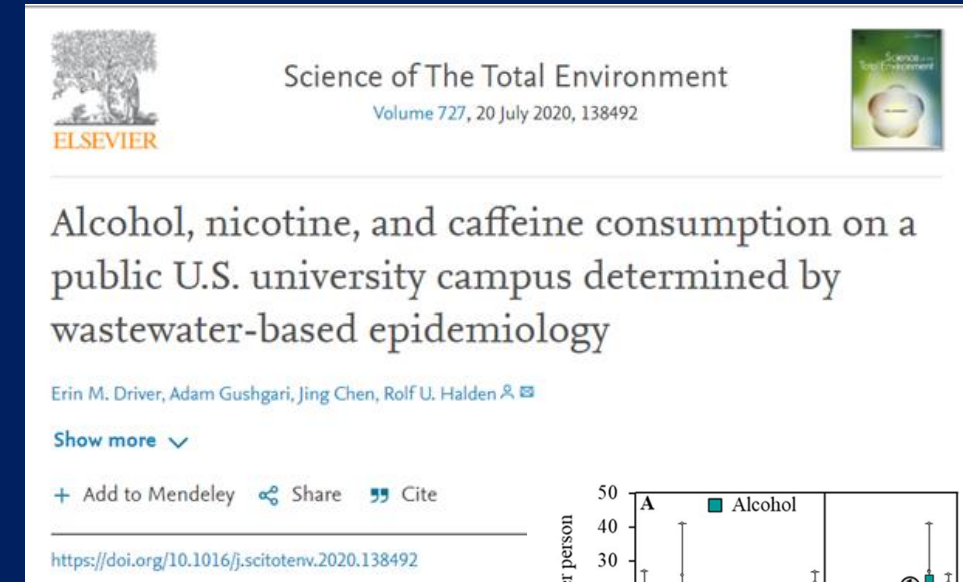
LC-MS/MS
Analysis

Data published from our lab

Estimation of substance use in the university campus via WBE

- First university-wide assessment of nicotine, alcohol, and caffeine consumption via WBE
- Estimates of per capita alcohol consumption agreed with self reported surveys
- Universities may benefit from the cost-effective WBE tool to manage health of students


Driver, E.M., Gushgari, A., Chen, J. and Halden, R.U. 2020. Alcohol, nicotine, and caffeine consumption on a public U.S. university campus determined by wastewater-based epidemiology. *Science of The Total Environment* 727, 138492.




Estimation of substance use in the university campus via WBE

- First reported detection of norfentanyl in U.S.
- Estimated heroin consumption exceeded national rate
- Estimated consumption of Attention deficit hyperactivity disorder (ADHD) medication was in-line with other U.S. campus studies


Gushgari, A.J., Driver, E.M., Steele, J.C. and Halden, R.U. 2018. Tracking narcotics consumption at a Southwestern U.S. university campus by wastewater-based epidemiology. *Journal of Hazardous Materials* 359, 437-444.




Journal of Hazardous Materials
Volume 359, 5 October 2018, Pages 437-444



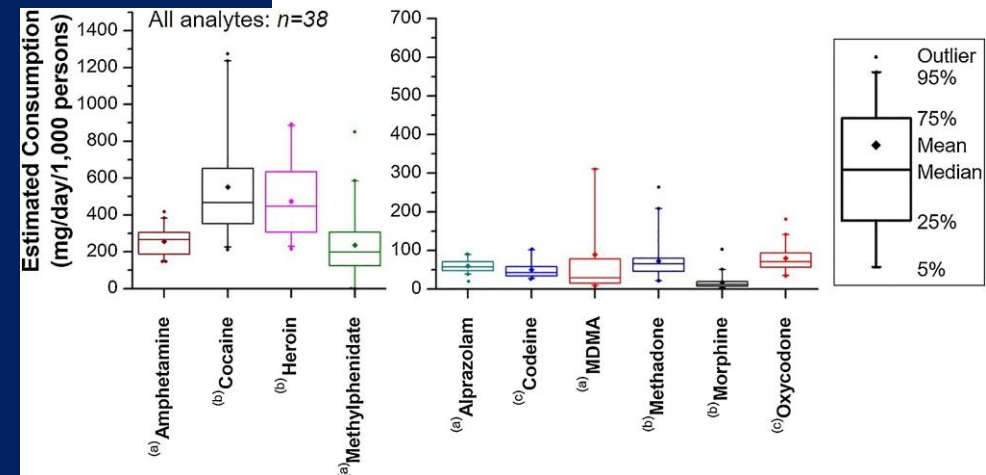
Tracking narcotics consumption at a Southwestern U.S. university campus by wastewater-based epidemiology

Adam J. Gushgari, Erin M. Driver, Joshua C. Steele, Rolf U. Halden 

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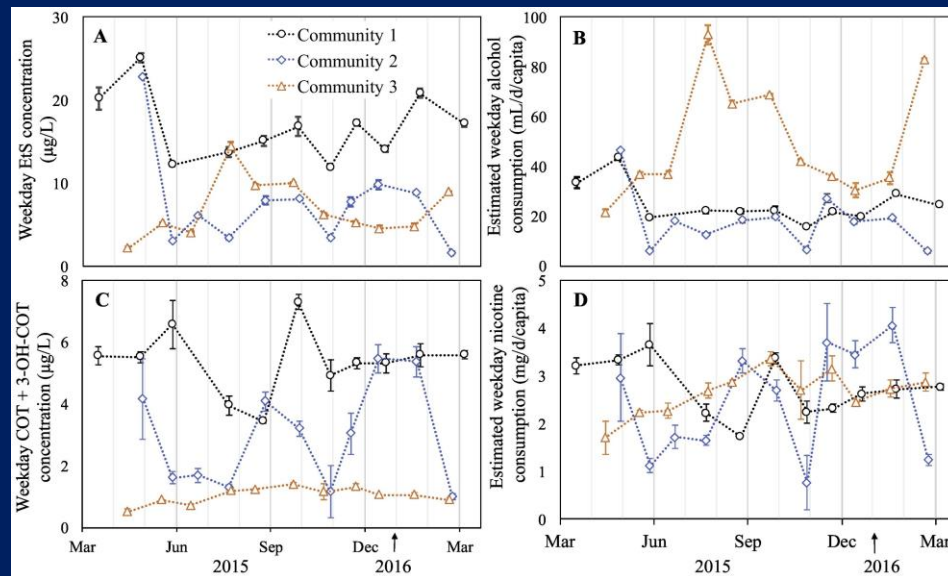
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<https://doi.org/10.1016/j.jhazmat.2018.07.073> [Get rights and content](#)
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Estimation of substance use in the U.S. communities via WBE

- First wastewater-based assessment of drinking and smoking habits in 3 U.S. cities
- Wastewater-based estimates were in good agreement with survey statistics



 Science of The Total Environment
Volume 656, 15 March 2019, Pages 174-183 

Alcohol and nicotine consumption trends in three U.S. communities determined by wastewater-based epidemiology

Jing Chen ^{a, b}, Arjun K. Venkatesan ^{a, 1}, Rolf U. Halden ^{a, 2, 3}

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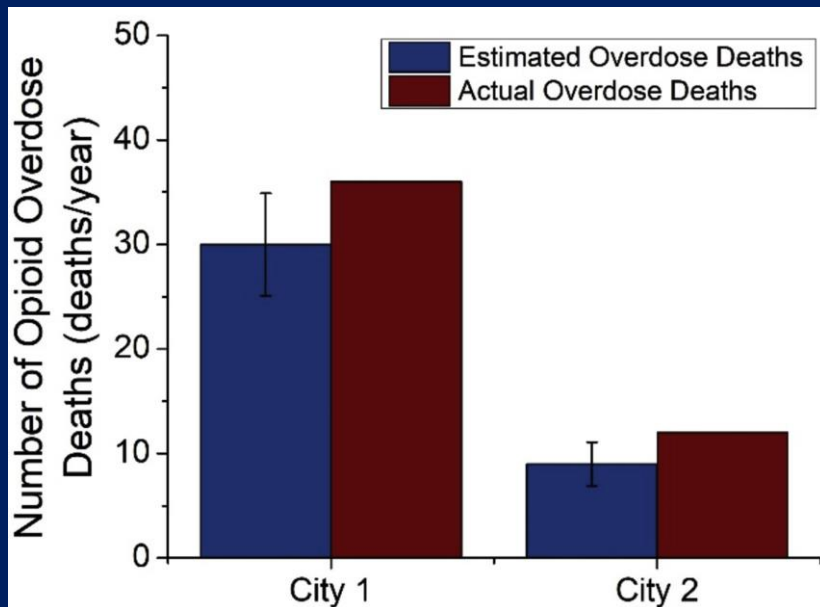
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Chen, J., Venkatesan, A.K. and Halden, R.U. 2019. Alcohol and nicotine consumption trends in three U.S. communities determined by wastewater-based epidemiology. *Science of The Total Environment* 656, 174-183.

Estimation of substance use in the U.S. communities via WBE

- First estimation of opioids overdoses and deaths from WBE data.



Water Research
Volume 161, 15 September 2019, Pages 171-180

ELSEVIER

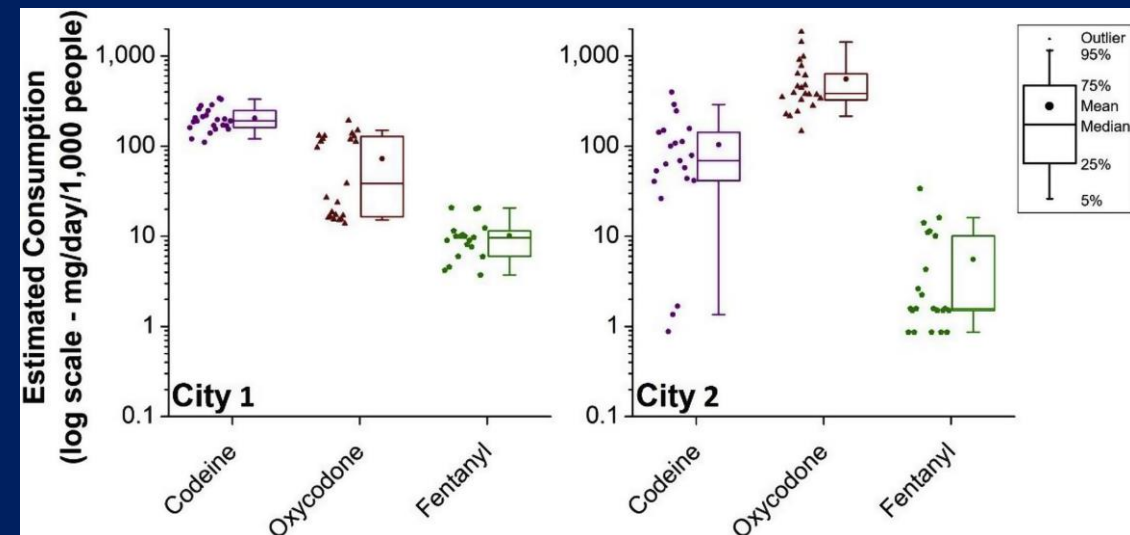
Long-term tracking of opioid consumption in two United States cities using wastewater-based epidemiology approach

Adam J. Gushgari ^a, Arjun K. Venkatesan ^b, Jing Chen ^a, Joshua C. Steele ^a, Rolf U. Halden ^a

Show more

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<https://doi.org/10.1016/j.watres.2019.06.003> [Get rights and content](#)



Gushgari, A.J., Venkatesan, A.K., Chen, J., Steele, J.C. and Halden, R.U. 2019. Long-term tracking of opioid consumption in two United States cities using wastewater-based epidemiology approach. *Water Research* 161, 171-180.

Dietary status using WBE

- First application of a WBE approach for monitoring dietary trends in two U.S. communities.
- Measured phytoestrogens (plant-derived human biomarkers) in community wastewater

Assessing the Potential To Monitor Plant-Based Diet Trends in Communities Using a Wastewater-Based Epidemiology Approach

Arjun K. Venkatesan, Jing Chen, Erin Driver, Adam Gushgari, and Rolf U. Halden*

DOI: 10.1021/bk-2019-1319.ch010

Publication Date: June 24, 2019

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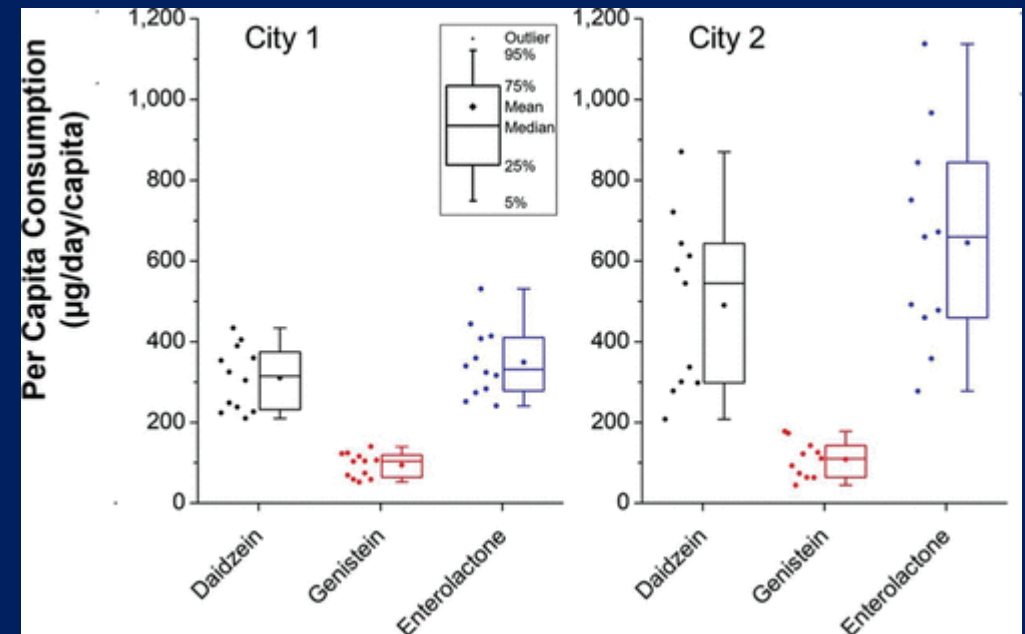


Wastewater-Based Epidemiology: Estimation of Community Consumption of Drugs and Diets
Chapter 10, pp 187-198

ACS Symposium Series, Vol. 1319

ISBN13: 9780841234413 eISBN: 9780841234406

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Venkatesan, A.K., Chen, J., Driver, E., Gushgari, A. and Halden, R.U. (2019) *Wastewater-Based Epidemiology: Estimation of Community Consumption of Drugs and Diets*, pp. 187-198, American Chemical Society.

Fast scanning mass spectrometer for population health surveillance

- **Short LC-MS/MS run**
- **Better economy**
- **Less labor intensive**
- **More signature chemicals in single run**

Collaborative data from an international study

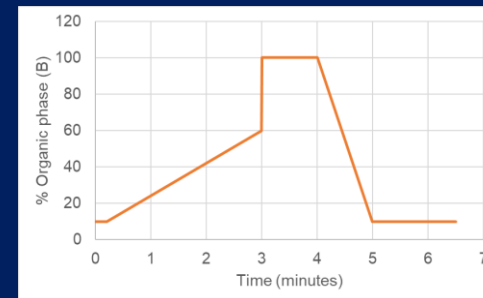
Methods

- 24-hour composite community wastewater samples collected from the UK, USA, and Mexico.
- Samples filtered through membrane filters and directly injected to LC-MS/MS for the analysis
- Guard column alone was used for separation of analytes



LC parameters

- HPLC column used- Biphenyl guard column (5.0 × 3.0 mm, 2.7 μm particle size)
- LC used- Shimadzu Nexera™ X2 ultra-high-pressure LC
- MS used- Shimadzu LC-MS 8060
- Mobile phases- Ultrapure water (A) and a mixture of acetonitrile and methanol (50:50,v/v) (B), (0.1% formic acid added in mobile phases for pH control)
- Optimized injection volume- 10 μL
- Optimized gradient





MS parameters

Nebulizing gas flow	3 L/min
Heating gas flow	10 L/min
Interface temperature	300°C
DL temperature	250°C
Heat Block temperature	400°C
Drying gas flow	10 L/min
Maximum dwell time	20 ms
Minimum dwell time	1 ms
Maximum loop time	0.572 ms

Results

- MS scan speed 30000 amu/sec
- Polarity switching speed of 5 ms
- 260 injections per 24 hours
- 135 analytes including pesticides, PPCPs, illicit drugs and their metabolites analyzed in 5 minutes
- 56 compounds quantified



Journal of Hazardous Materials
Volume 398, 5 November 2020, 122933

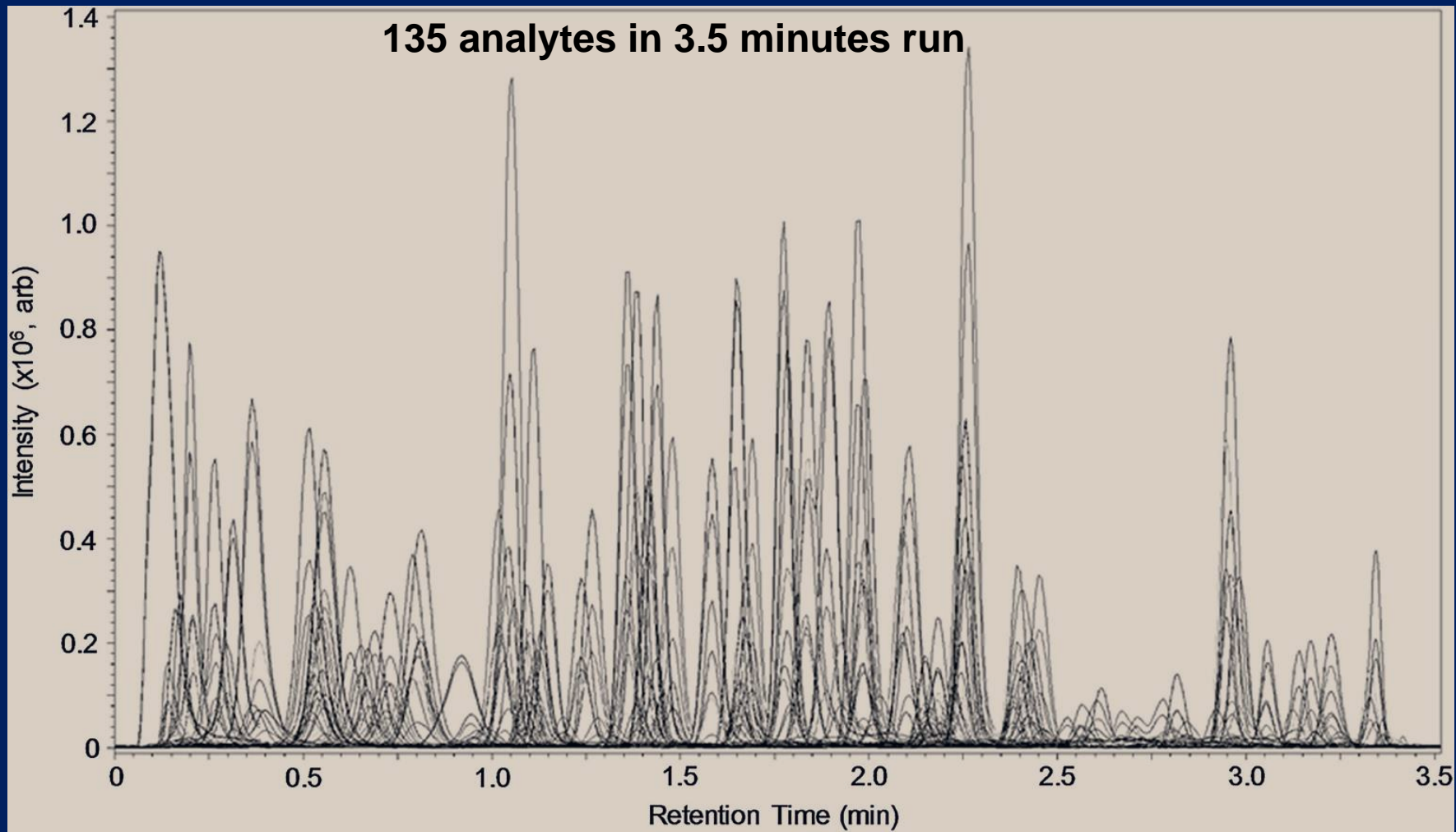
High-throughput multi-residue quantification of contaminants of emerging concern in wastewaters enabled using direct injection liquid chromatography-tandem mass spectrometry

Keng Tiong Ng ^{a, 1}, Helena Rapp-Wright ^{a, b, 1}, Melanie Egli ^a, Alicia Hartmann ^{a, c}, Joshua C. Steele ^{d, h, j}, Juan Eduardo Sosa-Hernández ^e, Elda M. Melchor-Martínez ^e, Matthew Jacobs ^b, Blánaid White ^b, Fiona Regan ^b, Roberto Parra-Saldivar ^e, Lewis Couchman ^f, Rolf U. Halden ^{d, h, i, j}, Leon P. Barron ^{a, g, k}

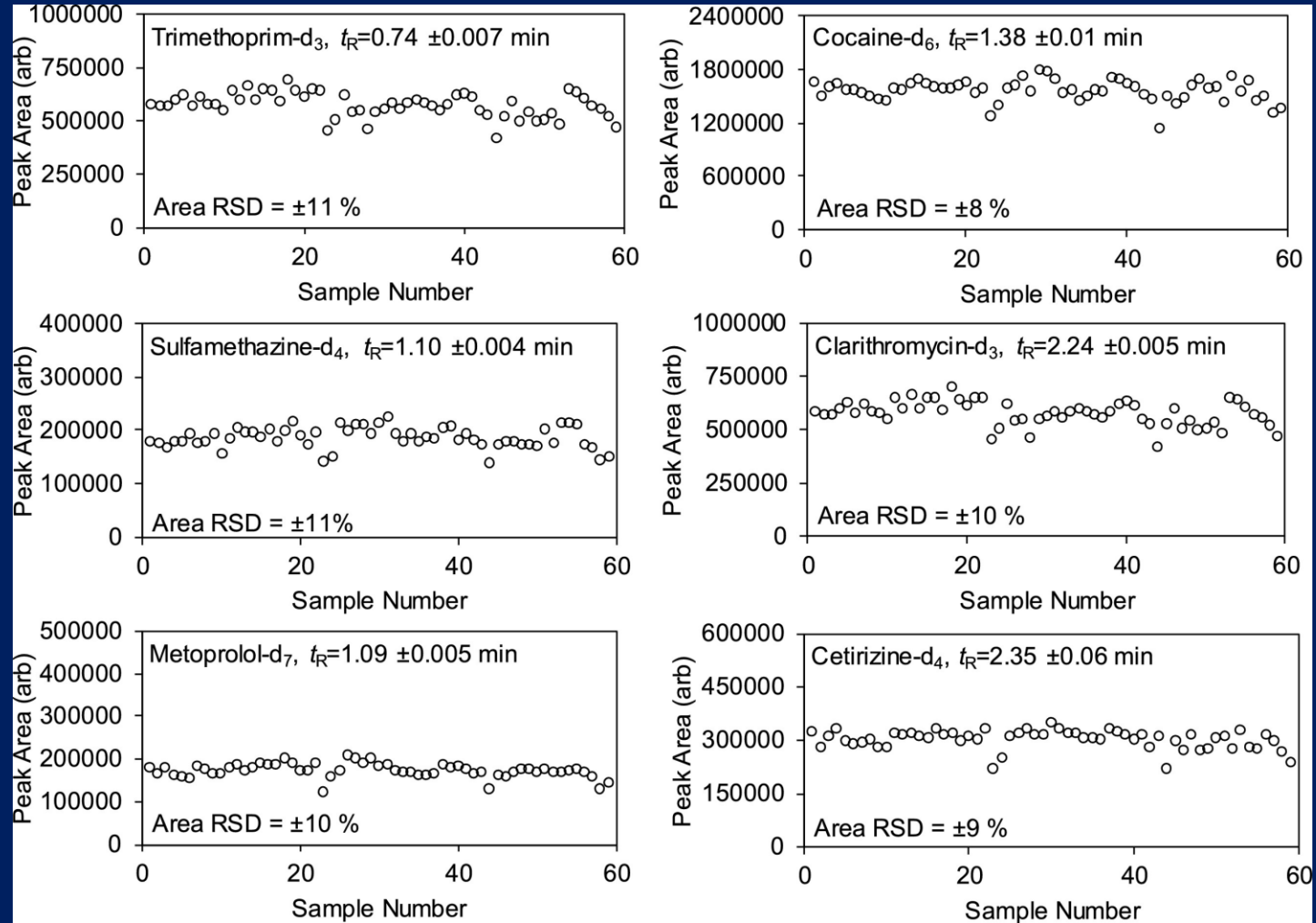
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Ng, K.T., Rapp-Wright, H., Egli, M., Hartmann, A., Steele, J.C., Sosa-Hernández, J.E., Melchor-Martínez, E.M., Jacobs, M., White, B., Regan, F., Parra-Saldivar, R., Couchman, L., Halden, R.U. and Barron, L.P. 2020. High-throughput multi-residue quantification of contaminants of emerging concern in wastewaters enabled using direct injection liquid chromatography-tandem mass spectrometry. *Journal of Hazardous Materials* 398, 122933.

Chromatograms of a mixture of CECs acquired by LabSolutions (version 5.93, Shimadzu)



Peak area and retention time stability



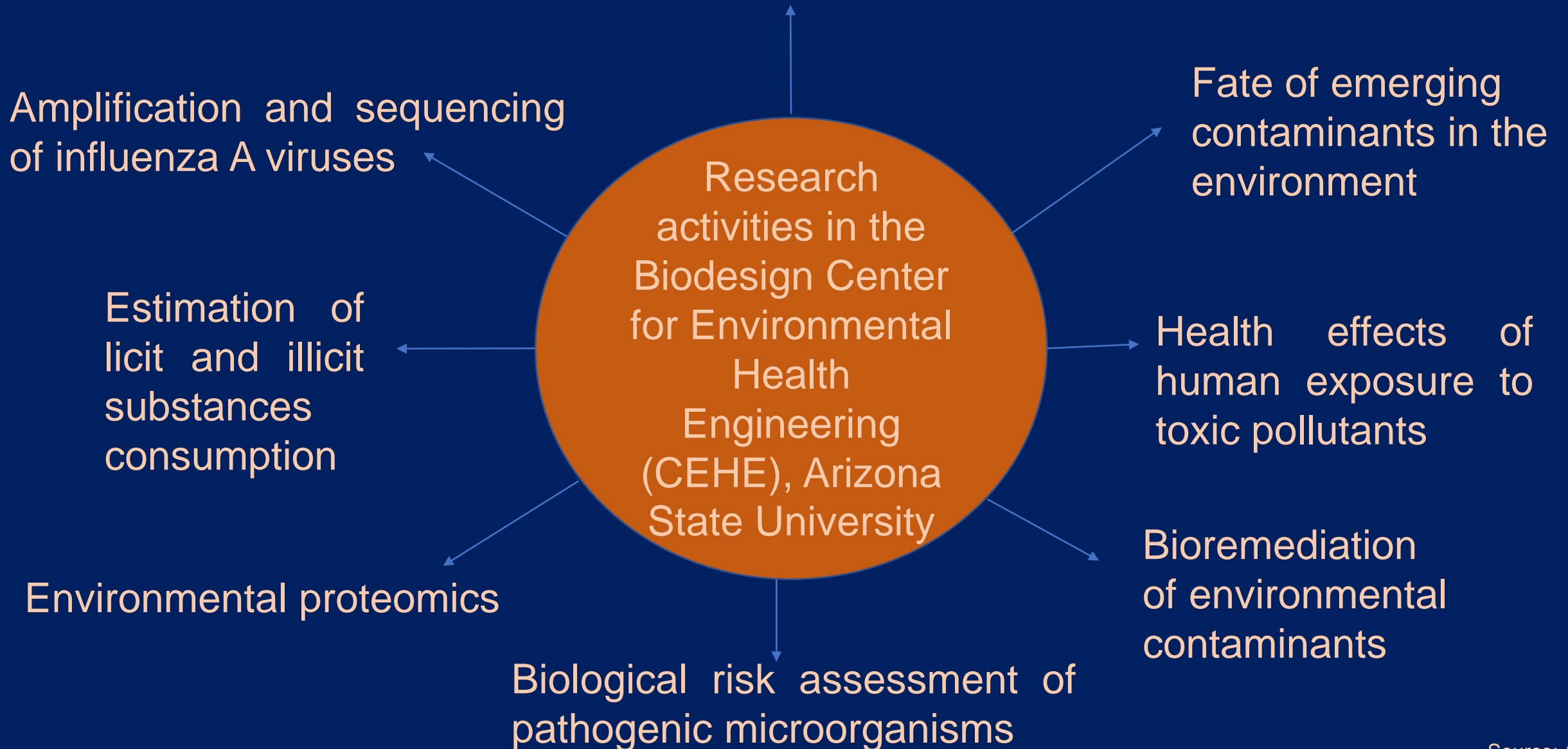
Analytical performance

	Linearity (R ²) (N>5)	Peak area precision (RSD %, n=6)		Matrix effect (% CV, n=6)		Inaccuracy (% CV)			Sensitivity	
		100 ng/L	1000 ng/L	100 ng/L	1000 ng/L	250 ng/L	750 ng/L	1000 ng/L	LLOD (ng/L)	LLOQ (ng/L)
Maximum	0.999	55	32	+337	+188	+66	+13	+9	533	1777
Minimum	0.967	2	1	-84	-60	-97	-54	-44	0.06	0.21
Absolute median	0.999	8	6	11	9	12	8	-4	9	31
Absolute mean (±Std dev)	0.998 (±0.0037)	11 (±10)	8 (±6)	20 (±34)	14 (±22)	16 (±14)	9 (±7)	-6 (±10)	29 (±55)	95 (±197)

Things to be aware of to ensure data quality

- Partitioning of analytes during sample filtration may have huge impact on what we measure
- A minimum of 20% loss of analytes featuring pH-dependent logarithmically transformed organic carbon-water distribution coefficient ($\log D_{OC}$) of ≥ 3.0 (Deo and Halden, 2010)

Public health well-being surveillance



Analytical services

- We are engaged in long-term, U.S. national monitoring with a growing network of over 220+ cities and community partners worldwide.
- We are being funded by sponsored research projects and by fee-for-service analyses.
- We analyze following biomarkers routinely under the umbrella of human health observatory (HHO).

Communicable diseases	Antitussives	Barbiturates
Benzodiazepines	Hallucinogens	Dietary indicators
Cannabinoids/Synthetic	Antibacterial/Antimicrobial	Alcohol and tobacco
Antidepressants	Parabens	Stimulants
Z-drugs	Opioids	Fentanyl and fentanyl analogues
Volatile organic compounds	Illicit drugs	Additional medications

Human Health Observatory (HHO) at Arizona State University ... An Early Warning System Established in 2006

- >500 WWTPs globally; >400 in U.S.
Representative of 15,000+ U.S. plants
Unbiased national estimates
- >12% of U.S. pop.; >40M people
- >250M people worldwide
- Largest archive in the U.S./world



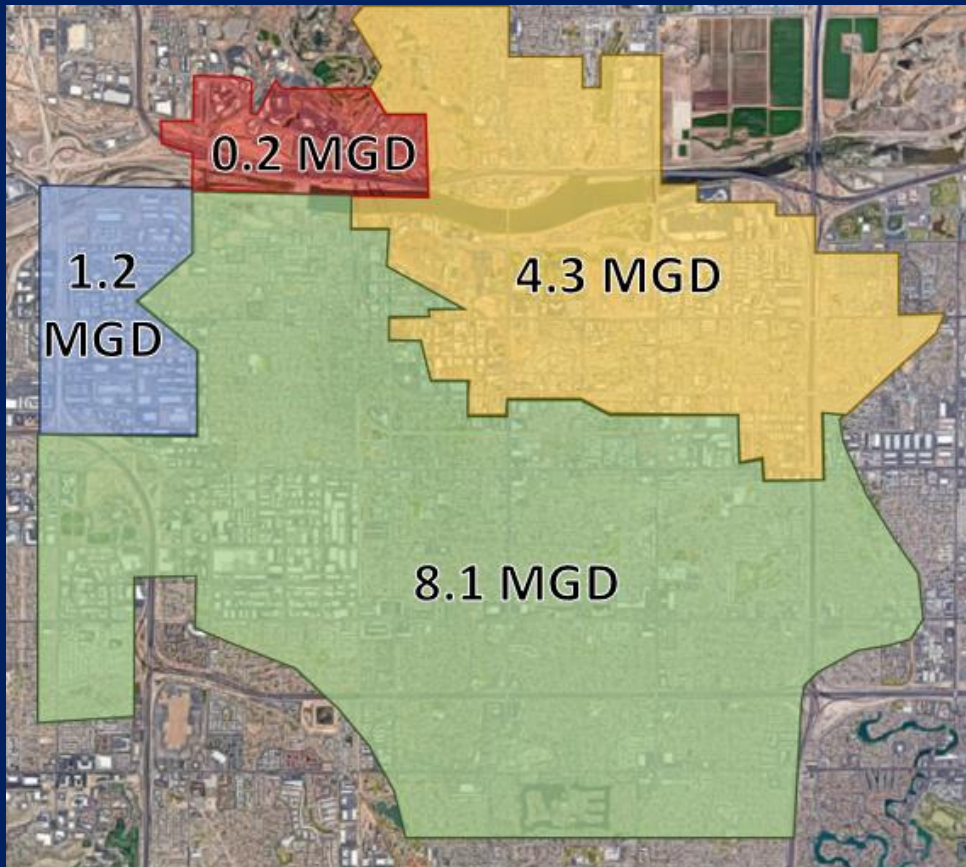
Environ. Sci. Technol. 2014. 48, 3603–3611

Venkatesan & Halden, 2014. *Environ. Sci. Pollut. Res.* 22 (3), 1577-1586

City of Tempe & ASU Collaboration



Center For
Environmental Health Engineering



- First U.S. municipality to use WBE data in strategy development and implementation.
- Addressing opioid addiction, marijuana use, and alcohol consumption.
- \$70,000 funding from City of Tempe and Arizona State University.
- City-wide capture of wastewater representing 185,000 contributing residents.
- Collaborative partnership with government, academia, industry professionals.



1st Public, WBE-informed, Opioid Online Dashboard Worldwide



Tempe Opioid Wastewater Collection Data...
Fighting Opioid Misuse by Monitoring Community Health

Collection Area Filter

- Area 1
- Area 2
- Area 3
- Area 4
- Area 5

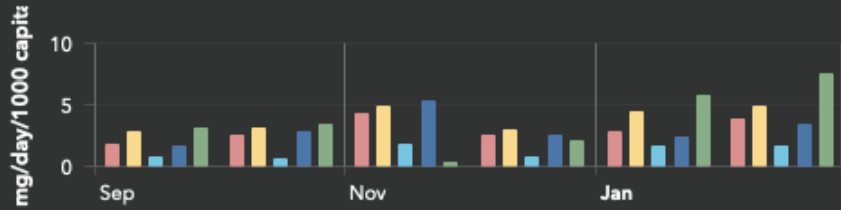
Date Range Filter 9/1/2019



4/14/2021

Please note that data updates have been paused due to COVID-19, but testing results will be updated in early 2021. Data is currently shown through February, 2020.

Fentanyl - opioid parent drug compound



Average Monthly Population Normalized Mass Load Value by Collection Area

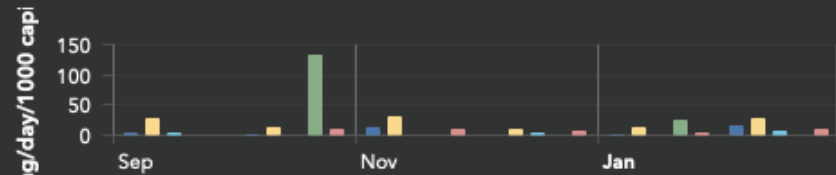
- Area 2
- Area 4
- Area 5
- Area 1
- Area 3

Fentanyl

Norfentanyl

Human Urinary Metabolite

Heroin - opioid parent drug compound



Average Monthly Population Normalized Mass Load Value by Collection Area

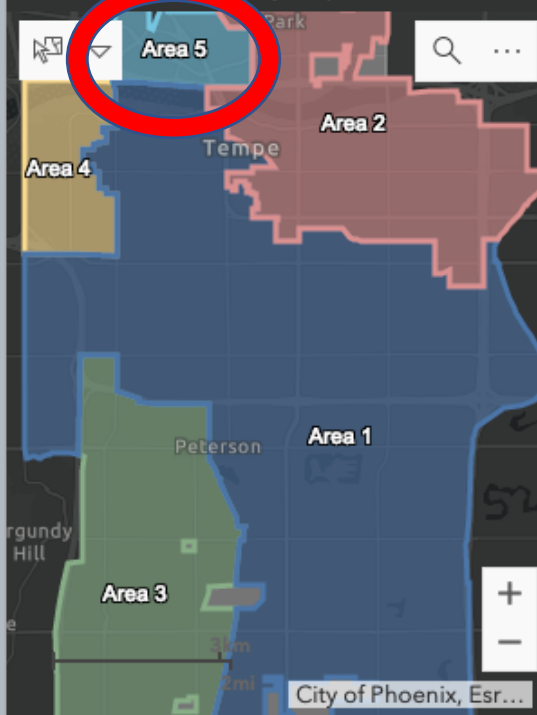
- Area 1
- Area 4
- Area 5
- Area 3
- Area 2

Heroin

6-acetylmorphine

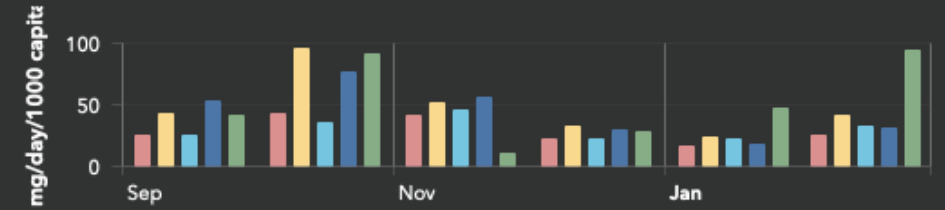
Collection Areas

(Click using map tools)



Data applies to entire collection areas and does not convey any information about specific neighborhoods or individuals.

Oxycodone - opioid parent drug compound



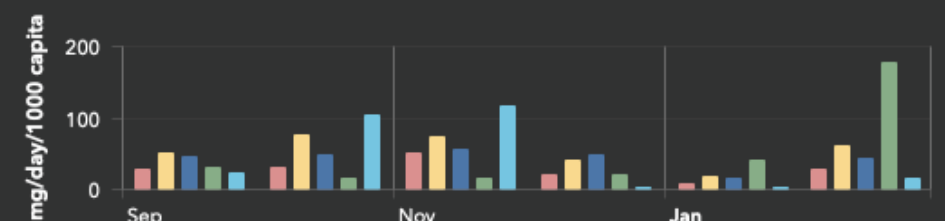
Average Monthly Population Normalized Mass Load Value by Collection Area

- Area 2
- Area 4
- Area 5
- Area 1
- Area 3

Oxycodone

Noroxycodone

Codeine - opioid parent drug compound



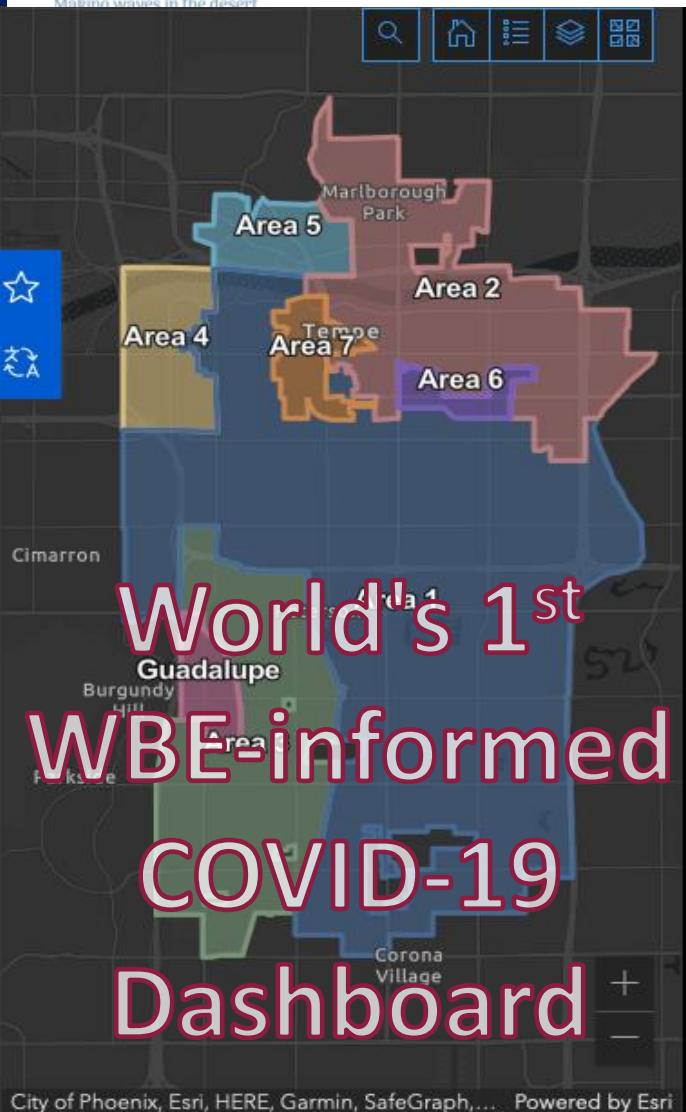
Average Monthly Population Normalized Mass Load Value by Collection Area

- Area 2
- Area 4
- Area 1
- Area 3
- Area 5

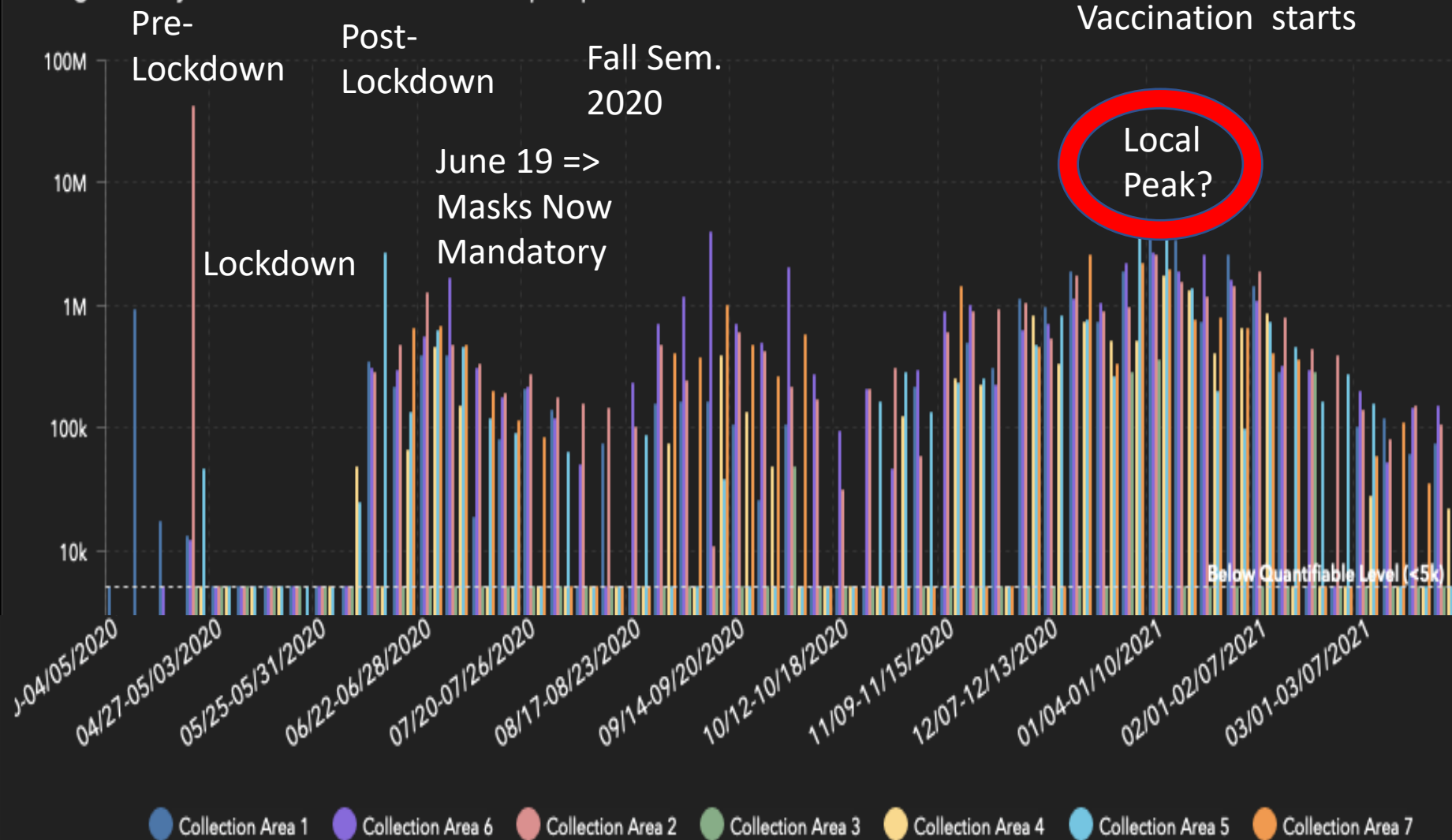
Story Map (How wastewater measurements are done): <https://arcg.is/PKWuz>

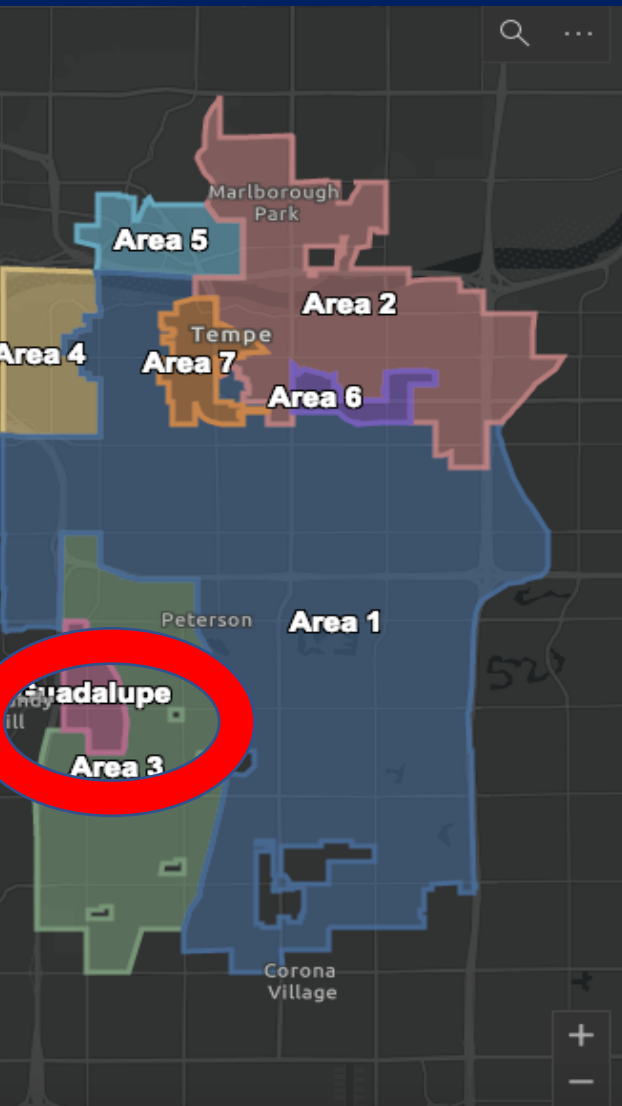
Dashboard (Opioid consumption in Tempe at the neighborhood level): <https://arcg.is/ey0Ha>

Developed 2018; Launched 2019

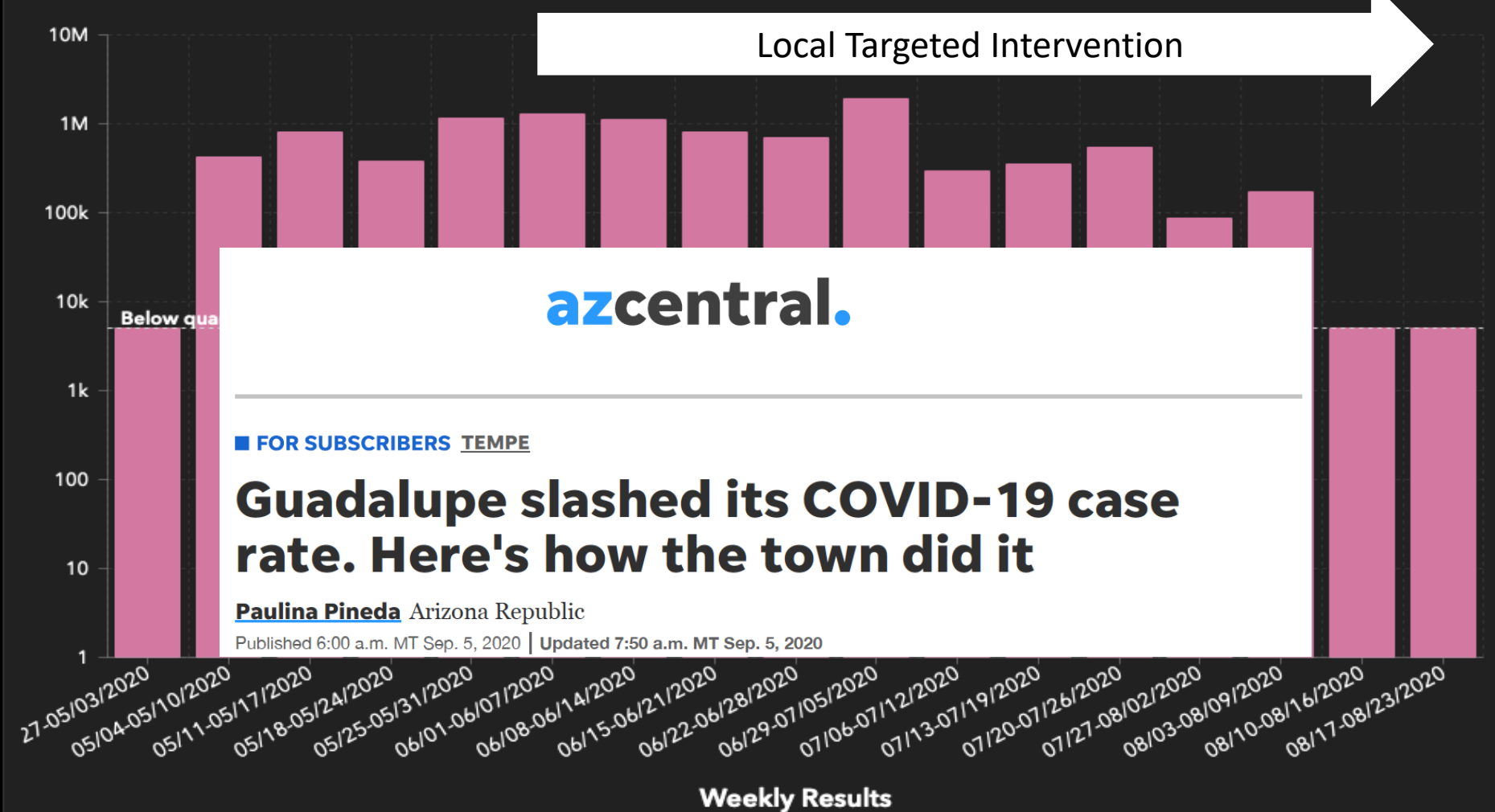


Average Weekly Results COVID-19 Genome Copies per Liter of Wastewater



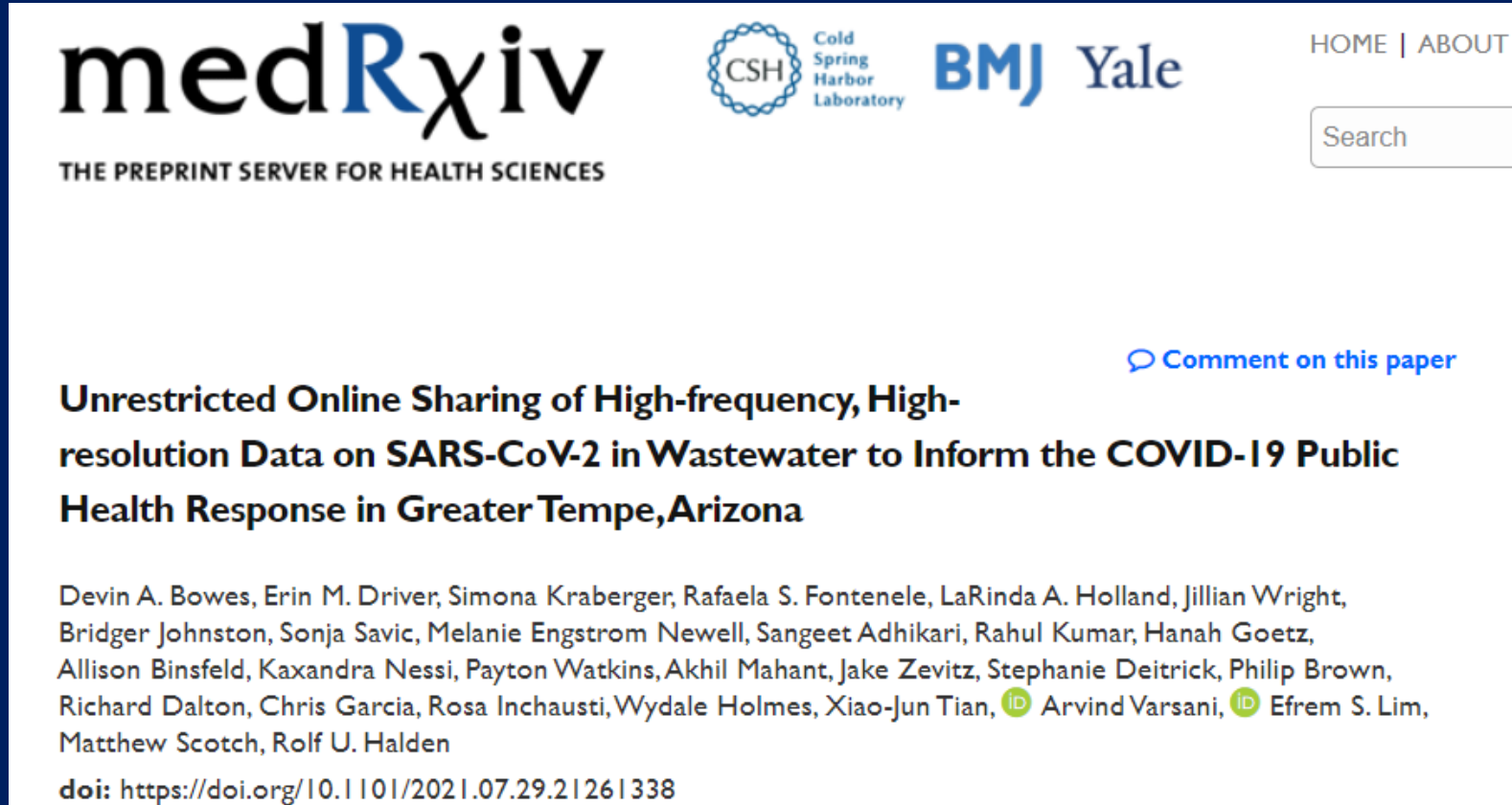


Average Weekly Results COVID-19 Genome Copies per Liter of Wastewater (Guadalupe)



A logarithmic scale (or log scale) is a compact way of showing numerical data over a very wide range of values

Recent publication of our COVID-19 dashboard



The screenshot shows the top section of a medRxiv preprint page. The medRxiv logo is on the left, with the tagline 'THE PREPRINT SERVER FOR HEALTH SCIENCES'. To the right are logos for CSH Cold Spring Harbor Laboratory, BMJ, and Yale. Further right are navigation links for 'HOME | ABOUT' and a search box. The main title of the preprint is 'Unrestricted Online Sharing of High-frequency, High-resolution Data on SARS-CoV-2 in Wastewater to Inform the COVID-19 Public Health Response in Greater Tempe, Arizona'. Below the title is a list of authors, including Devin A. Bowes, Erin M. Driver, Simona Kraberger, Rafaela S. Fontenele, LaRinda A. Holland, Jillian Wright, Bridger Johnston, Sonja Savic, Melanie Engstrom Newell, Sangeet Adhikari, Rahul Kumar, Hanah Goetz, Allison Binsfeld, Kaxandra Nessi, Payton Watkins, Akhil Mahant, Jake Zevitz, Stephanie Deitrick, Philip Brown, Richard Dalton, Chris Garcia, Rosa Inchausti, Wydale Holmes, Xiao-Jun Tian, Arvind Varsani, Efreem S. Lim, Matthew Scotch, and Rolf U. Halden. A DOI link is provided at the bottom of the preprint information.

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Unrestricted Online Sharing of High-frequency, High-resolution Data on SARS-CoV-2 in Wastewater to Inform the COVID-19 Public Health Response in Greater Tempe, Arizona

Devin A. Bowes, Erin M. Driver, Simona Kraberger, Rafaela S. Fontenele, LaRinda A. Holland, Jillian Wright, Bridger Johnston, Sonja Savic, Melanie Engstrom Newell, Sangeet Adhikari, Rahul Kumar, Hanah Goetz, Allison Binsfeld, Kaxandra Nessi, Payton Watkins, Akhil Mahant, Jake Zevitz, Stephanie Deitrick, Philip Brown, Richard Dalton, Chris Garcia, Rosa Inchausti, Wydale Holmes, Xiao-Jun Tian,  Arvind Varsani,  Efreem S. Lim, Matthew Scotch, Rolf U. Halden

doi: <https://doi.org/10.1101/2021.07.29.21261338>

Media coverage for Biodesign Center for Environmental Health Engineering

azfamily.com
POWERED BY CBS 5
NEWS COVID-19 WEATHER TRAFFIC GOOD MORNING AZ NEWS TIPS
109°F
12 WEATHER ALERTS

Groundbreaking study finds opioid drugs in Tempe wastewater system

Feb 02, 2019

RYAN SIMBUS
POSTED FEB 1, 2019

2 mL FENTANYL OXYCODONE NDC 0409-9093-32
Injection, USP only
1 mg Fentanyl/2 mL (0.05 mg/mL)

Get breaking news emails
Let Arizona's Family (powered by 3TV and CBS 5) keep you up-to-date on breaking news. We'll send it right to your inbox.

Researchers at A.S.U. turned to sewage water to find out how much opioids make it to Tempe's sewage system.

FOX 10 PHOENIX
News Coronavirus Weather Live Video Email Seen on TV More

Excessive Heat Warning

until THU 8:00 PM MST, Lake Havasu and Fort Mohave, Northwest Deserts, Lake Mead National Recreation Area, Parker Valley, Kofa, Yuma County, Central La Paz, Southeast Yuma County, Gila River Valley

"I love Prodigy because my kids love it, and they don't even realize they're learning math."
2nd Grade Teacher, North Dakota, USA ★★★★★

Testing wastewater for coronavirus: ASU researchers notice spike following end to lockdown

June 30, 2020

By John Hook | Published June 30, 2020 | Coronavirus in Arizona | FOX 10 Phoenix

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Biodesign receives \$1.5 million to develop early warning system for flu outbreaks

July 24, 2019

U.S. News
A WORLD REPORT

Autopsies Show Microplastics in All Major Human Organs

Researchers found evidence of plastic contamination in tissue samples taken from the lungs, liver, spleen and kidneys of donated human cadavers.

"We have detected these chemicals of plastics in every single organ that we have investigated," said senior researcher Rolf Halden, director of the Arizona State University (ASU) Biodesign Center for Environmental Health Engineering.

ONE WATER HEALTH

ASU Foundation Nonprofit Project

*A Nonprofit Project of the ASU Foundation:
“We are dedicated to serve and protect vulnerable
communities and environmental quality”*

STATES ACTIVELY PARTICIPATING IN COVID-19
WASTEWATER TESTING WITH OWOH



Near real-time monitoring
of environmental toxins



Conduct population-level
human health assessments



Seek opportunities to
close the material loop

Monitoring Toxic Exposures of Vulnerable Populations

Published Data on >300 Analytes

- Heavy metals
- Perfluorinated compounds
- Antibiotic resistance genes
- Plastic
- Industrial pollutants
- Radionuclides
- Bacterial pathogens
- Viruses
- Other

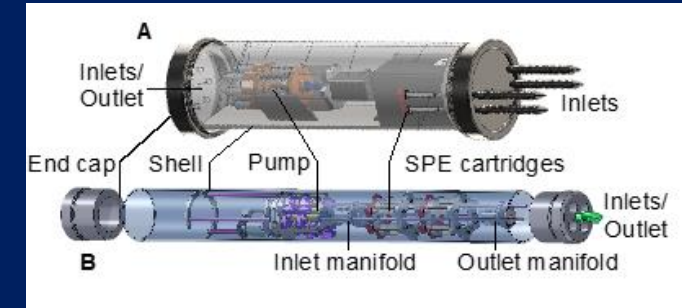


Dr. Adam J. Gushgari, PE
Chief Executive Officer

Water/Sewer Infrastructure Design
Chemical Wastewater Analysis
Data Modeling & Visualization



AquaVitas Presence & Repository Coverage



AquaVitas In-Situ (AV-IS)
Patented WBE Sampler

Lessons learnt

- Economic differences between cities impacts data quality;
- Lack of knowledge/education regarding what wastewater-based epidemiology is and how data can be used;
- Without field standardization (field, lab, & data analysis) significant variation in results can exist.

20+ Years Experience in WBE

200+ Peer-Reviewed Publications

Proven Methods of Population Health Assessment

Developed & Executed U.S. Department of Health & Human Services Wastewater Monitoring Program for SARS-CoV-2

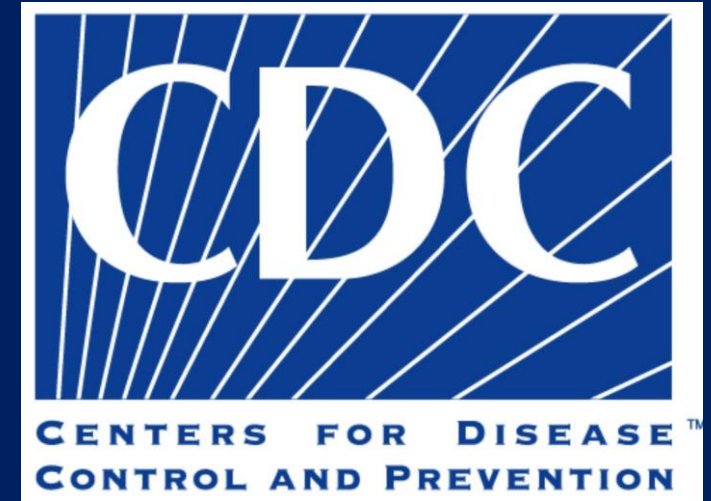
Has Served: U.S. Department of Health & Human Services, Local Government, State Government, Consulting Firms, Education, & Fortune 500 Companies

HHS US Nationwide COVID-19 WBE Monitoring – 320 City Phase

To potentially participate AT NO COST, contact: covid19@aquavitas.com

HHS & CDC Project

- Monitor 100 wastewater treatment plants & 10% of the U.S. population (Phase 1);
- Expand to 320 wastewater treatment plants & 30% of the U.S. population (Phase 2);
- Establish “best management practices” for wastewater surveillance efforts (Phases 1 & 2);
- Equip participants with wastewater data to manage pandemic locally (Phases 1 & 2)





THANK YOU FOR
YOUR ATTENTION



ANY QUESTIONS??