PROJECT WILDE

Wastewater and Illicit Drug Epidemiology – Preliminary Results

Nadine Harker | Mental Health, Alcohol, Substance Use and Tobacco Research Unit

Carrie Brooke-Sumner| Mental Health, Alcohol, Substance Use and Tobacco Research Unit

Pieter Venter| Biomedical Research Platform

Rabia Johnson| Biomedical Research Platform

Renee Street| Environment & Health Research Unit

Sizwe Nkambule| Environment & Health Research Unit

Noluxabiso Mangwana| Biomedical Research Unit

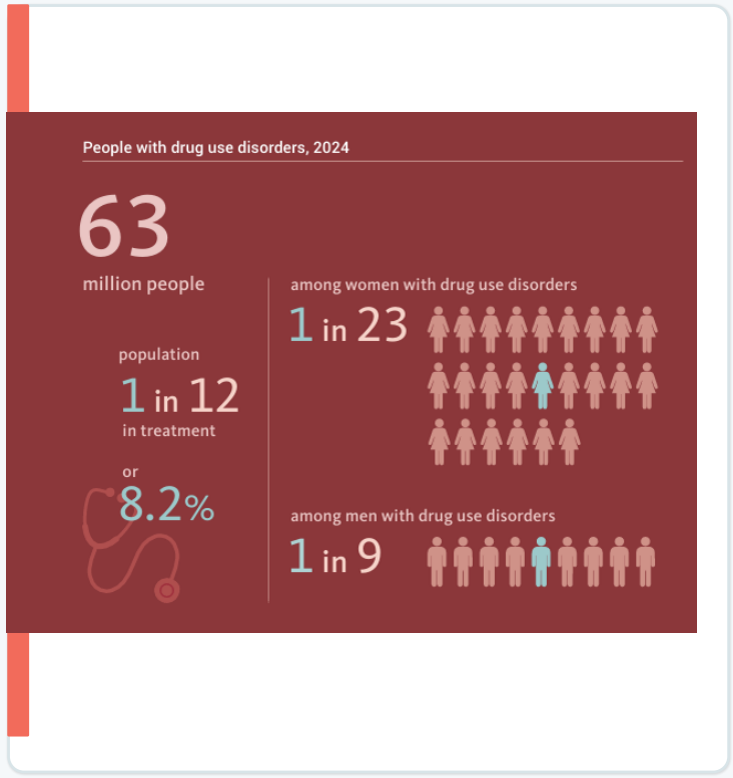
WESTERN CAPE PILOT

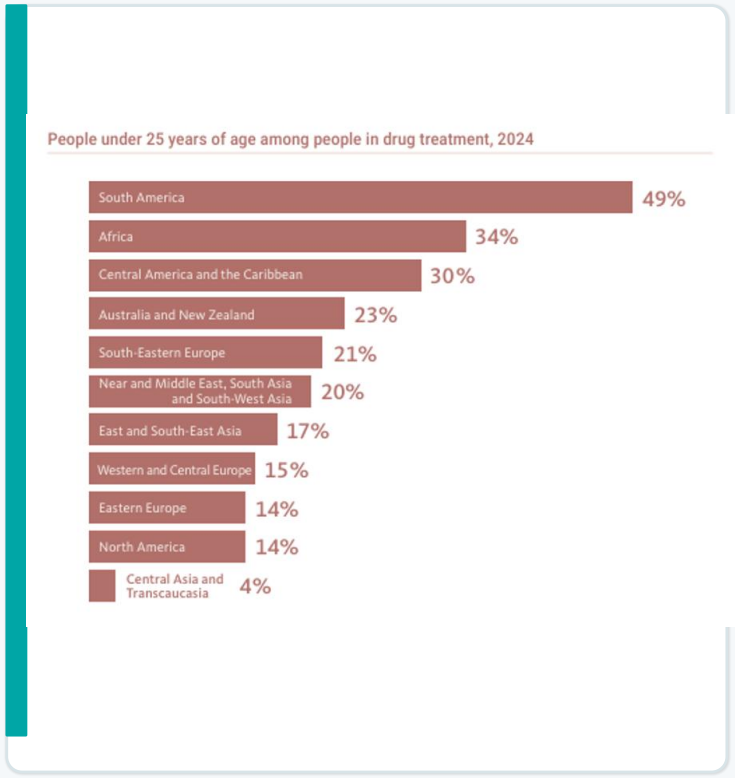




Background







Synthetic drugs are expanding rapidly - 755 new psychoactive substances (NPS) were identified globally in 2024, with 118 detected for the first time

www.unodc.org/unodc/en/data-and-analysis/world-drug-report-2026.htm

Why add wastewater-based epidemiology (WBE)?

- Drug use contributes to premature death, disability and the global burden of disease (Degenhardt et al., 2018).
- Associated infectious disease risks include HIV/AIDS, hepatitis and tuberculosis (NIDA, March 2022; Fazey, 1991).
- Other consequences include depression and anxiety, injuries, and social, family and economic strain (NIDA, March 2022; Fazey, 1991).
- Understanding drug-use trends and associated health factors helps assess health impacts and develop suitable interventions (Mutai et al., 2024).
- Public health surveillance helps monitor substance use and address its health impacts at a broader community and societal level.



BACKGROUND

Why add wastewater-based epidemiology?

.

Current surveillance

- Infrequent population surveys, crime, production and seizure statistics
- Service and harm-reduction data yield information on patterns of use, treatment need but not able to provide real-time data.

Persistent gaps

- Long delays between collection and reporting
- Under-representation of some populations which obscures results
- Limited ability to detect rapid changes
- Self-report and service-access biases

WBE contribution

- Objective population-level signal
- Non-intrusive measurement
- Near-real-time trend monitoring
- Triangulation with existing sources
- WBE data from Africa are scarce. A 2021 critical review of global WBE data noted that the method had not yet been applied across large parts of Africa (Water Res. 2021,

WBE complements rather than replaces established surveillance.

Study aim and objectives

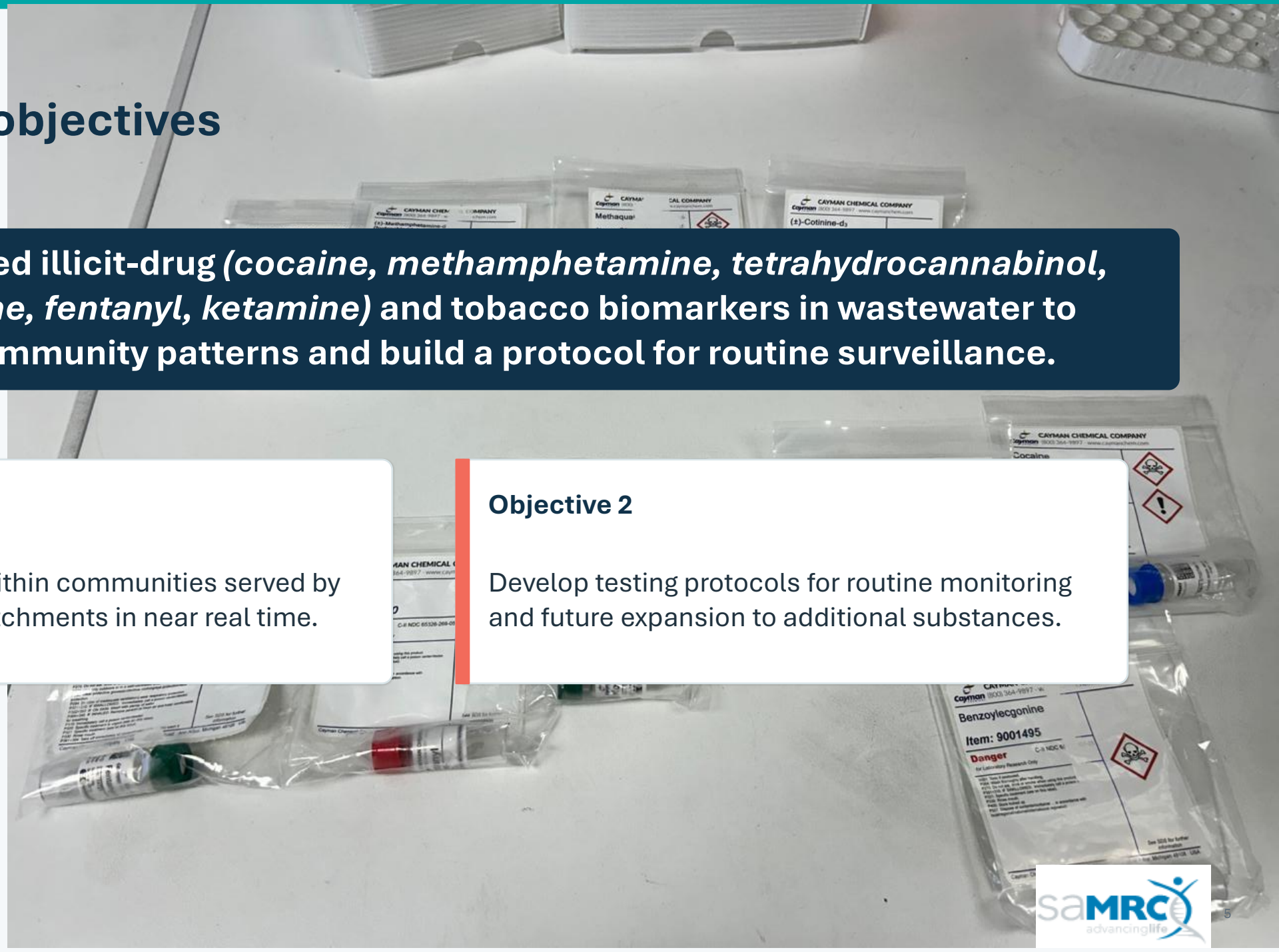
Monitor selected illicit-drug *(cocaine, methamphetamine, tetrahydrocannabinol, methaqualone, fentanyl, ketamine)* and tobacco biomarkers in wastewater to estimate community patterns and build a protocol for routine surveillance.

Objective 1

Estimate patterns within communities served by selected sewage catchments in near real time.

Objective 2

Develop testing protocols for routine monitoring and future expansion to additional substances.



Pilot design, governance and municipal approval

Study setting

Three municipal wastewater treatment plants in the Western Cape.
Of the 3, 1 WWP plant participated

Sewage Analysis CORe group — Europe (SCORE). SCORE was established in 2010 to standardise the approaches used for wastewater analysis and coordinate international studies through the establishment of a common protocol of action. https://score-network.eu/about/

City of Cape Town approval

Research approval PSRR-1382 was granted on 28 November 2025 for all sites, although the data represented reports on 1 WWP

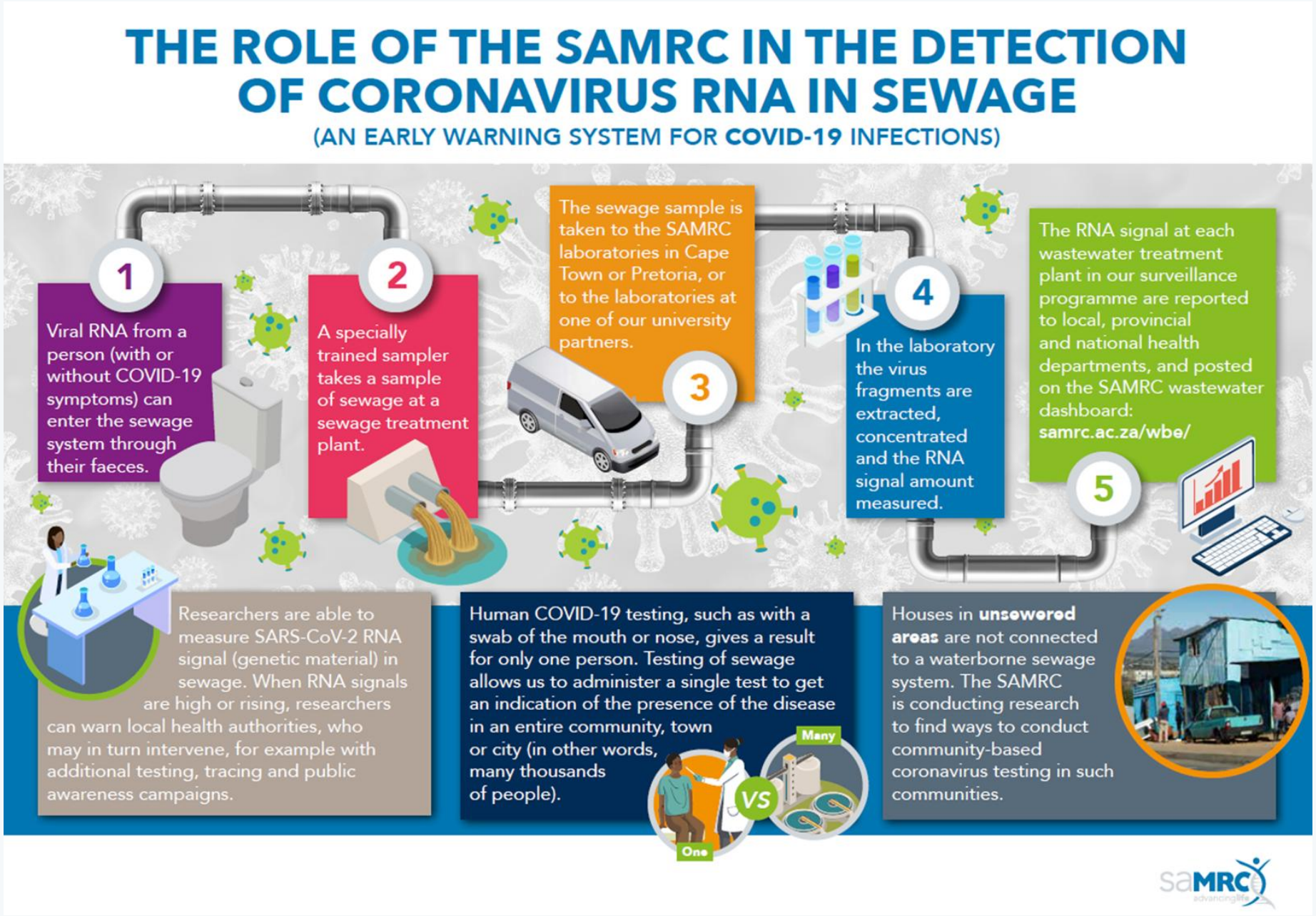
Plant locations are kept anonymous to protect the communities that fall under the selected WWTP catchment area.



1

Collect

Defined grab and composite wastewater sample



Wastewater sample collection:

- 24-hour composite sample
- ± 100 ml volume per hour
- ± 2.4 L homogenized sample
- Thursday-Friday every week
- Thursday-Sunday once a month
- Transported on ice

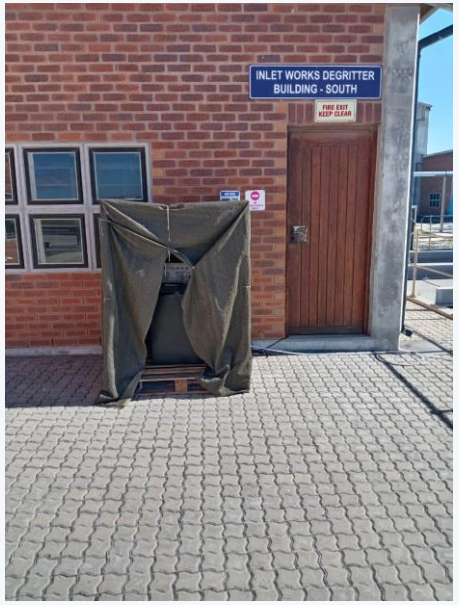




1

Collect

Defined grab and composite wastewater sample









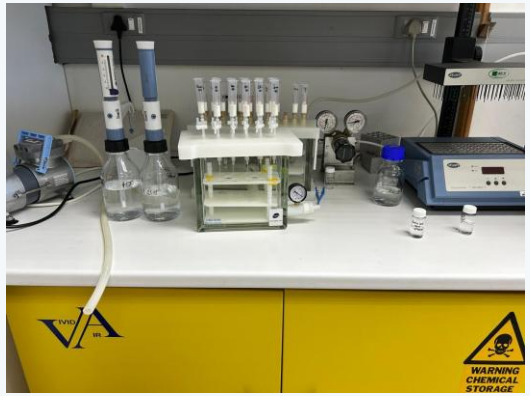


Sample Filtration and addition of internal standard



Sample extraction

Extract 40 ml of sewage influent using HLB cartridges



LC-MS/MS Quantification



Normalize

Adjust for daily flow and catchment population

Interpret

Compare trends across time

Outputs are community-level estimates and trend signals, not individual-level measures.

Analytical targets and quality control



Target drugs (Conc.)

- Methamphetamine (40 – 5000 ng/L)
- Nicotine marker: cotinine (300 – 40 000 ng/L)
- Ketamine (40 – 5000 ng/L)
- Cocaine markers: benzoylecgonine (40 – 5000 ng/L)
- Cannabis marker: tetrahydrocannabinol (40 – 5000 ng/L)
- Fentanyl and nor fentanyl (40 –5000 ng/L)

Quantification and Quality Control

- Eight calibration concentrations with internal standards
- Tap water used for calibrations curve
- Two MS/MS transitions per analyte for quantification and confirmation
- Raw-data processing in Sciex OS; further interpretation in Excel

Converting concentrations into comparable load estimates

Daily load

DL (g/day) = Concentration (ng/L) × Flow rate (ML/day) ÷ 1,000

Population-normalised daily load **

PNDL (mg/day/1,000 inhabitants) = DL × 10^6 ÷ Population estimate

- Reliable flow data and catchment-population estimates are essential.
- Population mobility, informal settlements and sewer-network coverage introduce uncertainty

Preliminary analytical results

- Results remain preliminary and should be interpreted with sampling and laboratory limitations.

What data is available

- Pilot samples (23 dated samples with 1 lost to splliage) have been analysed for methamphetamine, ketamine, fentanyl and cotinine.
- Ketamine and fentanyl were tested but not detected in the reported batch.

Quality

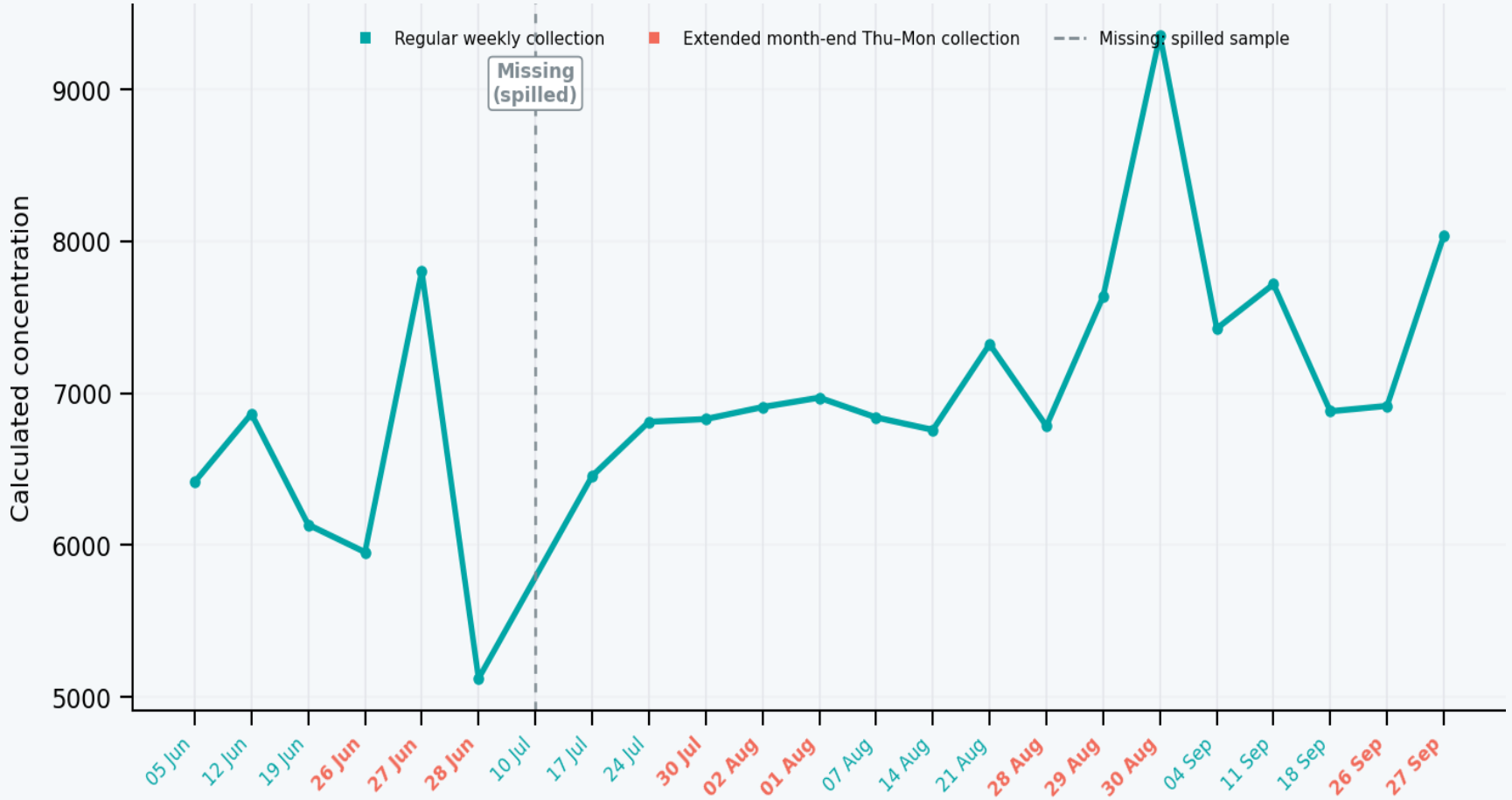
- One sample date missing data
- Remaining internal standards are needed for broader reruns and additional metabolites.
- Our analytical technique performed well and in line with SCORE-achieved 5 satisfactory results out of 6 (83.3%), with one questionable cotinine result and no unsatisfactory results.
- Precision and Accuracy attained

Early indication

- Higher methamphetamine concentrations in the pilot samples than the SCORE comparison samples.
- This is a concentration comparison

Cotinine concentrations

Calculated concentrations for 23 dated samples; extended month-end Thursday–Monday collection dates are highlighted in coral; regular weekly collections are teal.



Median

6,873

Highest dated value

9,357

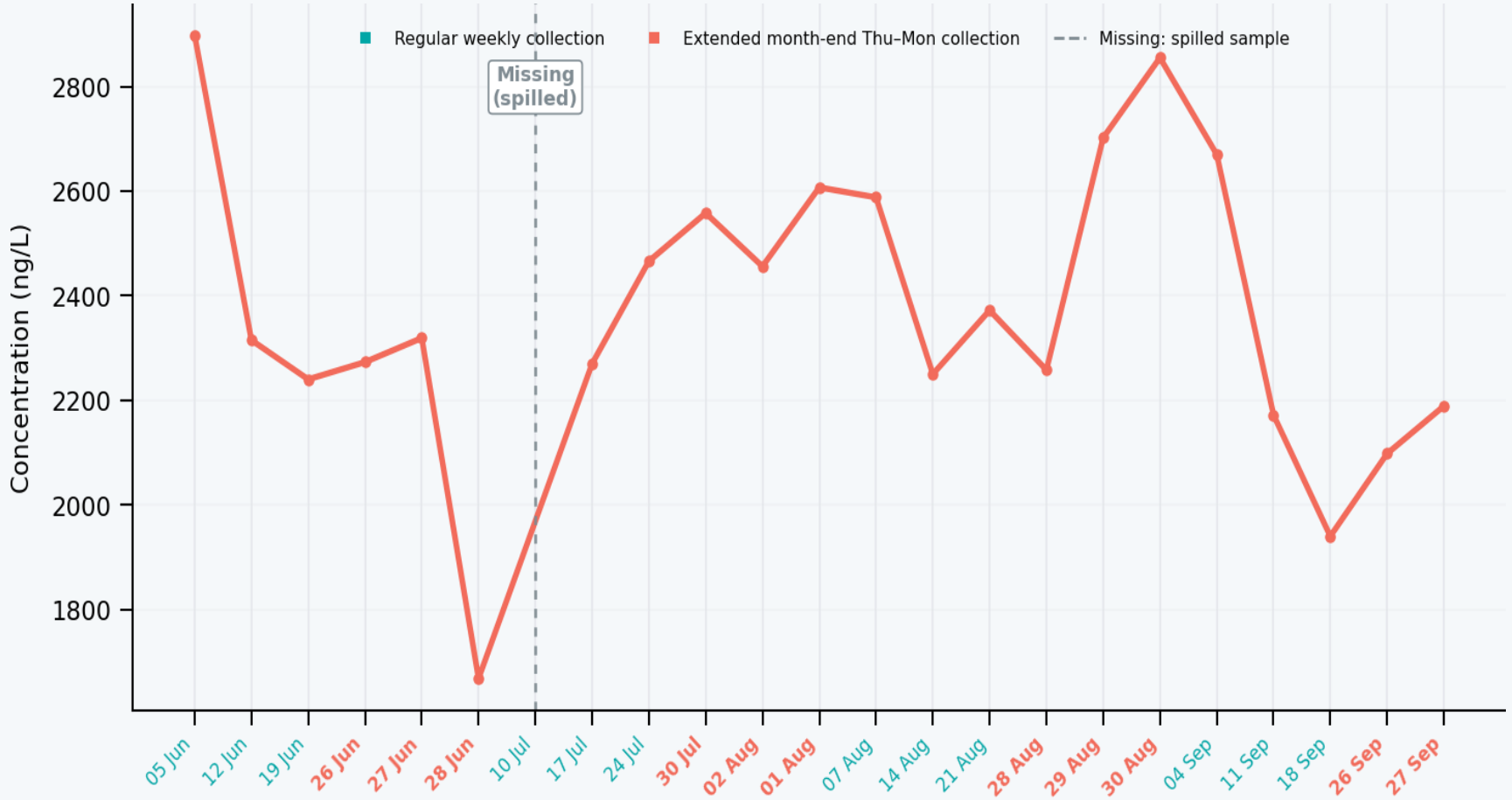
Lowest dated value

5,122

The 10 July date is retained in sequence as missing. The line connects the adjacent valid observations; no value is imputed.

Methamphetamine concentrations

Calculated concentrations in ng/L for 23 dated samples; extended month-end Thursday–Monday collection dates are highlighted in coral; regular weekly collections are teal.



Median

2,317 ng/L

Highest dated value

2,898 ng/L

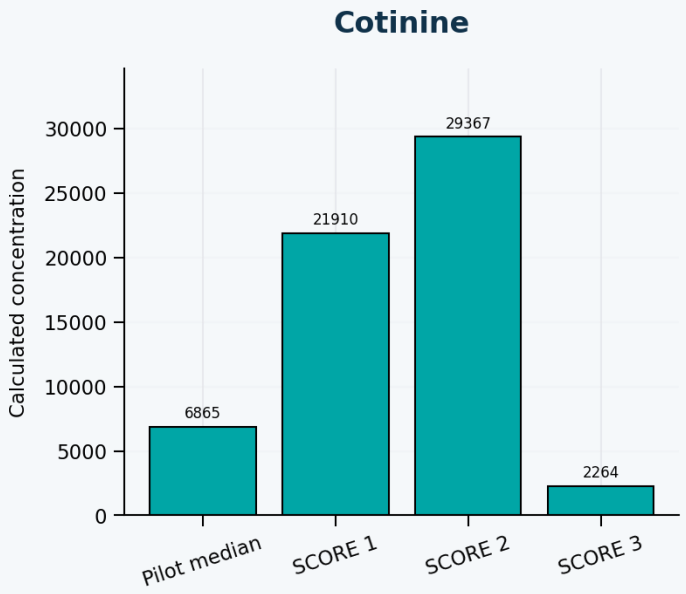
Lowest dated value

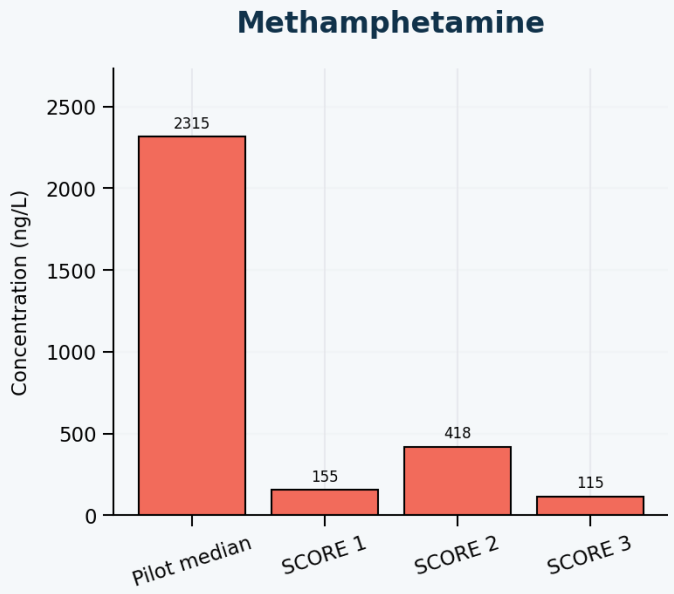
1,668 ng/L

The 10 July date is retained in sequence as missing. The line connects the adjacent valid observations; no value is imputed.

Analytical verification and prelim data using SCORE samples

SCORE samples were included to test the analytical method, not as direct comparators.





Purpose of the SCORE samples

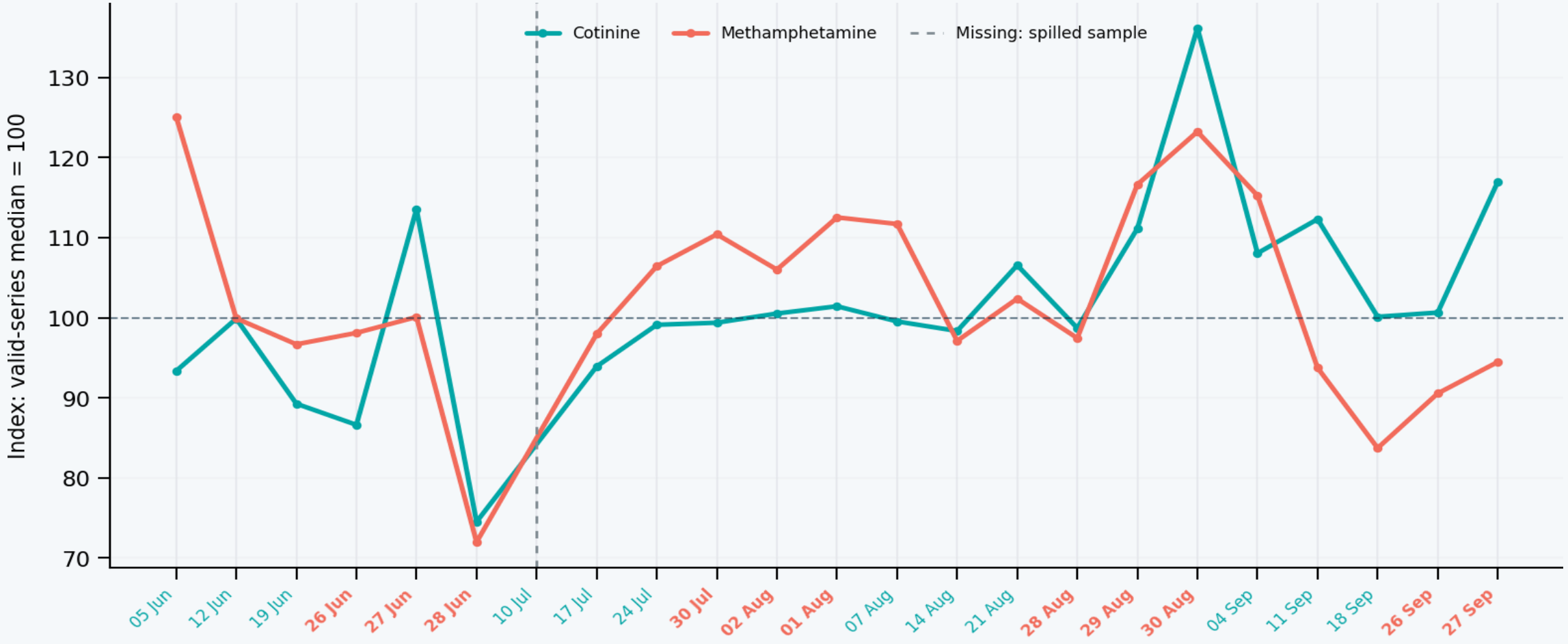
External samples used to test whether the analytical workflow could detect and quantify cotinine and methamphetamine across different concentration ranges.

Both target biomarkers were detected and quantified in the WILDE and SCORE samples, supporting the feasibility of the analytical approach.
methamphetamine median is far above all three SCORE samples.
Our cotinine level is below SCORE 1 and 2.

The samples were not collected as matched comparators and were not harmonised for sampling conditions, wastewater flow or catchment population.

Relative pattern across sampling dates

Each series is indexed to its own median (=100); extended month-end Thursday–Monday collection dates are highlighted in coral; regular weekly collections are teal.



Discussion

Analytical workflow works which demonstrates feasibility.

- Whilst we tested for fentanyl and ketamine, these were not detected.
- Methamphetamine was consistently detected, with some variation across dates and comparatively higher values during parts of late August. Consistent with Archer et al. - applied WBE at two South African treatment works in two provinces.
- Methamphetamine concentrations declined during September, reaching their lowest valid concentration since July (1,940 ng/L). Several law-enforcement operations targeting methamphetamine occurred in the Western Cape during September 2026, data do not permit attribution of this decline to specific seizures or enforcement activities.
- Cotinine was consistently detected and remained within a relatively stable band across most valid sampling dates, indicating a persistent nicotine-related wastewater signal. Consistent with international findings
- SCORE samples demonstrate that the analytical workflow can detect and quantify both biomarkers across different concentration ranges; they are not direct population comparators.

Limitations

- Current results are raw concentrations and have not been normalised for wastewater flow or catchment population. We will request flow data for our exact sampling dates and add population markers.
- Non-detection of fentanyl and ketamine does not mean no use. Fentanyl in wastewater might be found at much lower levels, so that cut-off may simply be too high – requiring adjustment.
- The sampling series is limited in duration and number of observations, restricting assessment of seasonality or longer-term change.
- Population mobility and informal settlements add uncertainty to any catchment estimate.
- Concentrations may be affected by wastewater dilution, rainfall, sewer conditions, analyte stability and sampling procedures.
- SCORE samples were collected for analytical-method testing and were not harmonised as epidemiological comparison samples.

Priority actions - Next steps



1. Strengthen field metadata + recruit additional WWPs
   Catchment-population estimates and calculate daily and population-normalised loads.
2. Confirm units, calibration performance, detection limits and quality-control results for each analyte.
3. Link each concentration to sampling duration, daily wastewater flow and relevant treatment-plant operational information.
4. Balanced weekday and weekend sampling – limited in our ability in separating a weekend effect.
5. Analyse the broader planned biomarker panel once standards and quality-control requirements are in place.
6. Analyse for new psychoactive substances (untargeted testing for NPS) that present as threats in other countries
7. Triangulate wastewater findings with other SACENDU data sources including seizure information.

Acknowledgements

Partners and enabling institutions

South African Medical Research Council (MAST-RU, BRIP, EHRU)

City of Cape Town Water and Sanitation Directorate

SCORE network (European Drug Agency) and collaborating wastewater-treatment personnel

This study is funded by MASTRU, BRIP and EHRU

Thank you