

Episode 87: Wastewater Analysis: Can Sewage Detect Emerging Drug Trends?

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Transcript

Hi everyone, welcome back to The Tox Lab. I'm Rebecca. I'm Rob. This week, we're going to be taking a look at something a little bit different.

What if I told you some of the most revealing data at a population level on drug use comes from a sample that everybody flushes down the toilet every day? Ooh. So I think it's fair to say I've been accused of talking shit on this podcast a few times. This week, we are literally doing that.

We're going to be diving, not literally, into the world of wastewater and having a look at what that data can tell us and how this unusual sample type can actually be really, really helpful in monitoring drug trends on a big level. Before we dive into today's episode, if you want to stay up to date with our content, you can find us on Instagram at @the_tox_lab. We also have a page on LinkedIn and we're fairly active over there. And for those of you who don't use social media but still want to get in touch with episode suggestions or comments on episodes, why not get in touch through our new website at thetoxlab.co.uk.

So wastewater sampling, and it is literally what it says on the tin. You go to a sewage treatment center, take some samples of essentially sewage, untreated sewage, before it gets processed, send it off to the lab, test it, easy, right? So there are a couple of different sampling methods that can be used for wastewater analysis when looking at drug trends. So you can use grab sampling, which is a single discrete sample that's collected at a specific time representing the wastewater conditions at that time.

And then there's something known as composite sampling, which involves mixing individual grab samples taken at regular intervals over an extended period. And this is usually about 24 hours. And this provides insight into the average wastewater over that time period. And once you've collected your wastewater sample, it goes off to the lab.

The lab probably do a bit of cleanup using a bit of solid phase extraction, and essentially extract any drug or drug residue, drug metabolites from that sample and measure the concentration. Yeah. And just to sort of complete the picture, thinking back earlier on in the process, the logic is a person, or rather lots of people within a given area, take drugs, those drugs pass through their body, through their excreta into the sewage system. You just pulled the best phase.

I did. So that is the logic in a nutshell of wastewater sampling. And for big area data, it's great, right? It tells you what is happening across a big population.

So it's really helpful, not for monitoring an individual person. I mean, it's utterly useless at that, but it's really good at monitoring larger macro trends. Are lots of people using cocaine on a Friday night? Are lots of people using ketamine now more than they were three years ago?

It's those big questions within a geographical area. So why don't we dive into our first paper this week? So in 2021, the UK wastewater analysis programme was launched in partnership with the Home Office, the National Crime Agency and the Defense Science and Technology Lab, or DSTL. And the aim of this was to monitor drug misuse across England using wastewater-based epidemiology.

So the first phase of this work was to establish an in-depth baseline of drug use in England across 15 locations measured at a high temporal resolution, so sampling at least once every two days. And the four objectives of this work to qualitatively monitor 20 prioritised illicit drugs and cutting agents in wastewater samples taken across 2022 using both direct injection and SPE-LC-MS methods to investigate the consumption trends and correlations and to identify any impact of public holidays, large events and law enforcement seizures on drug consumption. So let's just break down some of those big words.

Direct injection is mass spec without any cleanup or chromatography whatsoever. Yeah, I think so. Really, really dirty, but letting your mass spec do the work. The other one was the method I described where you use solid phase extraction, and then you just use typical LC-MS, triple quad, cleaner analysis.

Now, interesting point about this paper is we don't actually know where those 15 locations are, do we, other than they're in the UK? Yeah, they're spread, I think, fairly evenly across the UK, across England rather, but they don't state the specific locations of the wastewater treatment plants that they sampled from. So, samples from 15 wastewater treatment plants spread across England with population equivalents ranging from 100,000 to 3.5 million people, and the population equivalent coverage was estimated at 1.8 million or 21% of England. Okay, so a massive chunk of the population then.

Yeah, there were 1,746 wastewater samples representing 360 separate days, and 18 out of the 20 target analytes were detected and quantified, and eight substances were detected in every sample, and these were BZ-E, cocaethylene, cocaine, EDDP, ketamine, lidocaine, methadone, and morphine. So, in this work, they look at population normalised load, which is the daily mass load of whatever you're looking at divided by the catchment population, and I might refer to it throughout this as PNL. And the PNL was relatively consistent and was highest for BZ-E with an average of 773 milligrams per 1,000 people per day.

When looking at ketamine, seven catchments had a higher PNL on average than all other international wastewater treatment plant catchments, which suggests that in those areas, lots of ketamines being used way more than a lot of Europe. Okay, that's an interesting stat. Cocaine PNLs were considered high for eight catchments. Three catchments had high PNLs for MDMA and amphetamine, and one catchment had a high PNL for methamphetamine.

It's a shame we don't know where these catchments are, because that would be really interesting information. Yeah, and both ketamine and cocaine had statistically significantly higher weekly mean values in England compared to other European sites, which are participating in a network over the same sampling period. So it does seem to be that in England, ketamine and cocaine are being used quite frequently. That's the suggestion then, that there's more ketamine and cocaine use in England relative to the wider studies being carried out in Europe.

Yeah, and it did take a look at quite a few cocaine adulterants, including fenestil, procaine, and benzocaine, and these were detected at low frequencies across all sites, whereas lidocaine, which is another adulterant, was quantifiable in all samples ranging from 10 nanograms per liter to 2,576 nanograms per liter. But it's important to note that lidocaine can be legitimately prescribed to treat sore throats and mouth ulcers, so it doesn't necessarily represent cocaine use. There are other legitimate reasons for it being there. That's fair.

I mean, there are also other legitimate reasons for ketamine. This is also true. Being somewhere, yeah. So in the study, they did take a look at iced tinnitosis and fivamino iced tinnitosis, which were just starting to emerge in the drugs plight in 2022, and these were not detected in any samples.

The limit of detection for the method was 10 nanograms per liter, and because these compounds are so potent with very small doses that are also being diluted in wastewater, this might mean that the concentration is below the limit of detection. Iced tinnitosine, interestingly, also apparently suffers from quite severe matrix effects in wastewater, making it even harder to detect, not even accounting for how little there will be in wastewater, and the stability of iced tinnitosine in wastewater hasn't really been studied, and might also be an issue. I can't imagine it's that stable. No.

It's quite lipophilic, and I'm not surprised that they struggled to detect iced tinnitosine, although this was 2022. Yeah. So I don't know. In fact, we don't know.

We've no idea how much iced tinnitosine was around in 2022. Yeah, but it's interesting that they added it to those 20 drugs that they wanted to look for, so. Yeah, no. And it would have been a finding if they had detected lines, right?

So probably worth looking for. So moving on to looking at temporal trends, population normalised daily consumption did vary between the different catchment areas that they were looking at. Amphetamine, ketamine, MDMA, and methamphetamine consumption was relatively stable throughout 2022. Heroin consumption decreased in May of 2022, but then steadily increased until the end of the year.

And the timeframe did coincide with the announcement of the ban in opium poppy cultivation in Afghanistan, which happened in April. That's interesting. Yeah. So there was a very short drop in that ban window.

I mean, we subsequently now know with the power of hindsight that really the heroin hasn't yet gone away. Yeah. But interesting that there was a sort of supply shock just through the announcement. Yeah, I guess there was like a redistribution in how much people were wanting to sell so they had supply.

And it's interesting that trend could be put to them wastewater. Yeah, yeah, yeah, definitely. This sort of shows the power of it, doesn't it? Cocaine consumption was significantly higher in Q2 and Q3, and there was a noticeable drop in consumption in March, 2022.

And on the 17th of March, 3.7 tons of cocaine were seized at the Southampton docks, which does coincide with this drop in cocaine consumption. And the eight sites that did drop were back up to average P&L levels by April. And it's hard to prove causation in this situation, but large drug seizure at port, and then apparently a relatively consistent drop off in cocaine use, presumably the logic being, this is a supply shock effectively. Yeah.

There were also quite clear recreational use trends at weekends for cocaine and MDMA with a peak appearing on a Sunday. No surprises there. And elevated drug consumption was observed over bank holidays, and this was approximately 21% higher than regular Fridays and Mondays. On Halloween, there were two sites that had outliers for consumption of amphetamine, one site for cocaine, ketamine and methamphetamine, and three sites for MDMA.

So there seemed to be a spike on Halloween in certain catchments for these drugs. Is Halloween a traditional amphetamine related celebration? I don't know. That's really, really odd.

Is it just people having parties? Probably. And maybe so many people use cocaine all the time that it wasn't sensitive enough to also spot a spike in cocaine. Seems an odd finding though.

Yeah. And there were 68 outliers that were found during the period of the World Cup, which took place at the end of 2022. We know that big sporting events and big celebrations are associated with an increase in recreational drug use. So that fits as well.

Two sites also reported outliers for cocaine use that coincided with Eurovision, and one site for methamphetamine with three presenting outliers the following day for cocaine and MDMA, two sites presenting outliers for ketamine and methamphetamine. That's slightly more surprising. Yeah, I didn't think Eurovision would be a big... No, I had no idea there was a big drug culture surrounding Eurovision, but maybe I just live under a rock.

Maybe. And interestingly in this data, you can also see associations with local events. So one site had high cocaine consumption the day after a major football game that was held in the local area. Okay.

Heat waves also saw an increase in cocaine and MDMA use in a region representing a population equivalent to over a million. Right, okay. So I'm gonna push back on this slightly. Is it that drug use goes up in a heat wave?

Or is it that everybody's urine's just a bit more concentrated? I was thinking that, yeah. Although I don't know how the sewage system really works. Presumably whatever I put in a toilet is massively diluted by everything else.

So maybe it wouldn't be sensitive enough to detect that. Has anybody ever done wastewater creatinine? To look at trends in... I don't know.

I'd love to know whether that's relatively stable or whether that does oscillate. Yeah. You might not detect anything and therefore the conclusions of this paper on MDMA use are sound. So also a few correlations between drugs.

As expected, there was a correlation between the presence of parent drugs and their metabolites. They also looked at cocoethylene and BZP and L ratios. And these were significantly higher on the weekend which they suggested indicated the consumption of proportionally more alcohol than cocaine as part of higher recreational use during the weekends. Okay, yeah.

So relatively cocoethylene was higher at the weekends. There's two hypotheses. One is the same people who are taking cocaine the rest of the week are then drinking more. Or you've actually got two different populations.

You've got a group of people who regularly take cocaine on a daily basis. Yeah, and then you've got the people who go out on a weekend have a cup of drinks and take cocaine. Precisely. We can't tell those two populations apart, can we?

So I think this paper does a really good job of highlighting what wastewater is good for and the sort of trends you can see, the sort of little nuggets of information that you can derive from it. And it is a really, really powerful technique. I think it is important that you don't over interpret it. Yeah.

Because there can be sometimes multiple explanations for one finding, right? Yeah, of course. But it is helpful for sort of like for like comparisons geographically, comparing London to Amsterdam for example, can be really helpful. So these are the common drugs, the things that we're most likely to encounter in wastewater and probably in our day-to-day toxicology case work.

What about some of the novel drugs? You mentioned Isotenitazine and they didn't detect anything. Has anyone done MPS wastewater monitoring? So the next paper we're going to talk about is a paper out of Australia that was looking at wastewater collected from across Australia between August, 2024 and June, 2025 to provide a spatio-temporal perspective on the presence of xylazine and or nitazenes.

So they collected 24-hour composite samples representing the daily influent wastewater entering the plant. For capital city sites, 18 to 20 sites were sampled every two months and for regional sites, between 38 and 39 were sampled every four months. In total, there were 702 samples that were

analysed and 10% of the samples gave positive detections for one or more of the 11 nitazenes and 28% of the overall samples had xylazine present. 28%?

That's huge. I know. I mean, I think even 10% of all the samples being positive in nitazenes is quite impressive. Yeah, yeah.

Okay, well, let's dive into this a bit more and we can unpack some of that later. 57% of the sites with nitazene detections were also positive xylazine during the same collection. So it does seem to be that co-use trend. Yeah.

There were 88 nitazene detections of 10 different nitazenes across the positive samples. So the majority of samples only had one nitazene present but some had more than one nitazene present. And the 10 nitazenes that were detected were butynitazene, clonitazene, etodesnitazene, etonitazene, isotonitazene, metodesnitazene, metonitazene, N-piperidinyl etonitazene, N-pyrrolidino etonitazene and protonitazene. You've practiced saying those words before.

I have, yeah. What study period was this? August 2024 to June 2025. So what's surprising me here is a lot of those nitazenes were not ones that were particularly prevalent in the UK at that point in time.

No. Some of them had occurred a little bit earlier, early 21, 22. In April, 2025, there was a day where four nitazenes and these were etonitazene, metodesnitazene, isotonitazene, and butonitazene that were detected at quantifiable levels in one sample. When looking at spatial detections, capital city sites had nitazenes and xylazines detected in every state or territory with Western Australia and Victoria having the highest proportion of nitazene detections of 18% and 14% of samples respectively.

And the Australian capital territory had xylazine detected in 50% of the samples that were collected, which is mental. It is, yeah, it is. nitazenes were also detected in every state and territory that had regional sites sampled with New South Wales, Northern Territory, and Tasmania having the highest detection frequency for nitazenes and South Australia and Victoria having the greatest detection frequency for xylazine at 49 and 37% respectively.

When looking at temporal detections, nitazene detections in capital city sites showed a general decline in nitazene detections from 15% of samples containing one or more nitazene in October, 2024 to 5% by June, 2025. Regional sites, interestingly, didn't see the same trend and had a detection frequency of between 7 and 11% across the three collections over that year. That's interesting. So it declined in capital cities.

But remains stable. But remains stable in more suburban areas. Yeah, it is important to note that the capital city sites were being sampled twice as often as the regional sites. So there might still be a bit of a trend that we're not capturing at these regional sites because they were only really sampled three times.

Okay, that's fair. The xylazine detection in capital cities is fluctuated with an increase being noted between August and October, 2024 with a decline to April, 2025 and then an increase in June, 2025 which coincided with the highest detection frequency of xylazine at 37%. And regional sites also showed a similar pattern. So while the nitazene trend seemed to be different between capital cities and regional sites, the xylazine trend seemed to be fairly consistent.

That's also interesting, isn't it? A lot to unpack here. And it's important to note that for the 69 samples when nitazenes were detected, only 12 samples had nitazenes above the limit of quantitation with concentrations ranging from 0.4 to three nanograms per liter which the equivalent to excreted amounts ranging from 0.02 to 1.63 milligrams per day per thousand people. Okay, so really, really, really tiny detections.

Yeah. Okay. And for xylazine at 69% of the positive samples had a concentration above the limit of quantitation which was set at 0.1 nanograms per liter with a range of 0.02 to 66 milligrams per day per thousand people. And that's quite a range and the detection of 66 milligrams a day per thousand people for xylazine was from a sample in August, 2024 and the metabolite four hydroxy xylazine was also detected helping to confirm that this was from human consumption rather than xylazine entering the wastewater supply via another route.

So first and foremost then, it shows that wastewater sampling can be helpful for novel drug detection. Yeah. And I'm impressed actually at the range of nitazenes they managed to detect across the wastewater sampling. Yeah.

I think it is important here when we see some of these numbers and we think all 50% positive for xylazine, things like that. This doesn't tell you how many people are taking these compounds, only that somewhere within that area, xylazine is entering the waterway. And thanks to the presence of metabolite, presumably we can conclude that that is through people. Yeah.

So we can say xylazine is entering the drug supply but we don't know to what extent only that it's widespread phenomena across Australia. In another paper looking at wastewater collections from 22 countries looking at nitazene detections over the new year period between 2022 and 2023 and 2023 and 2024, found a sample from Australia on the 30th of December, 2023, which was positive protein nitazene with a mass load of a hundred milligrams per day per thousand people, which is huge. And this declined significantly the following day.

I remember seeing this paper because I believe it made some headlines and then I looked at the numbers and I looked at the maths and I thought this doesn't sound right. And I think the authors do address that, don't they? Yep. So the samples were also screened for metabolites for hydroxine nitazene, five amino protein nitazene and endozethal protein nitazene.

And none of these were detected in that sample. So this indicated that the protein nitazene may have been directly disposed of into wastewater rather than being detected as a result of protein nitazene being excreted. And this takes us on to one of the bigger issues with wastewater sampling. You don't always know it's been through people.

Yeah, it's why looking for metabolites of the different drugs at the same time is so important. Yeah. And I assume it's not that uncommon for people to dispose of unwanted drugs down a toilet. Yeah, I feel like that's the first option.

You know, something like highly potent protein nitazene. Yeah. It does require a little bit of caution when looking at some of this data for that reason. But I did find this particular spike particularly interesting because it was huge.

Must have been a significant powder dump. Yeah, must have been to spike it to that level even with the natural dilution that happens with wastewater. So moving on to our final paper which was published very recently at the beginning of July, 2026 looking at N-propyl nitrile chlorthene or cyclothne in Tennessee. And this was detected in seven wastewater samples from one rural wastewater catchment situated along interstate 75 in Tennessee with measure concentrations ranging from 1.58 to 10.6 nanograms per liter.

And the normalized mass load ranged from 2.61 to 5.48 milligrams per day per thousand people. And this was comparable to the normalized mass load of metonitazene and N. pyrrolidino etanittosine that were detected in New Orleans during 2025. So again, proof of concept, a different class of novel opioids but the premise still works. You can detect cyclothne in wastewater as well.

They looked at 11 other wastewater sites and didn't detect cyclothne in any of these. But this may have been due to the sampling being collected in August, 2025 which was quite early on in cyclothne's emergence on the illicit drug market. It would have been, yeah. But it does show the power of looking at wastewater that you can pick up very like newly emerging novel drugs.

And that does play a role in early warning systems and harm reduction strategies too. I've just had a thought. Yep. A lot of people are doing this for triple quad.

Yeah. Because you need the sensitivity. But imagine we could do this with high res. Ooh.

Then we could go back in time and see when compounds first emerged in the wastewater samples. That would be really interesting. Yeah. That would be amazing.

Detect that first sample where a new drug emerged and then that would almost give other agencies a date to kind of go back and re-refuse data like post-mortem data where you're seeing that sort of trend from that time or not could be quite interesting. It would be, yeah, it'd be really interesting, wouldn't it? I don't know if you'll get the sensitivity on some of these instruments. Maybe you will with some of the modern ones.

Yeah, possibly. And I guess it depends on how you're prepping your sample. If you're like really concentrating it, then you've obviously got a better shot of detecting these novel opioids that are always going to be present at very low levels. That is actually a positive point here.

I don't know if you've ever been to a wastewater treatment works. Can't say I have. I went to one on a school trip many years ago when I was about 13. There is not a shortage of fluid coming in at the top end of the treatment centre.

So you're right. You can collect litres and litres of fluid and concentrate your sample up. I hate the word fluid in that centre. I don't like it.

I mean, that's what we're talking about. So we just have to be grateful about it. So as well as this data being quite a broad representation of what's going on in a certain catchment area, this data might be quite valuable for early warning systems and harm reduction. It's likely that this data is probably available quicker than some of our other sources of data, like post-mortem data that a lot of the time has to be like posting quest, for example.

It might play a really valuable role in those systems of making sure that when we detect a new novel drug that harm reduction strategies are put in place, other agencies are alerted so they can start looking for it and things like that. Real-time monitoring could allow you to spot hotspots of increases in drug use fairly quickly, couldn't it? For example, if there's suddenly a spike in ketamine in a town somewhere, that could be a useful piece of information. This original paper we looked at was 15 sites around England.

And I get the feeling it was quite involved work. Scale that up to every wastewater treatment plant in the country. And I could see merit in that data, but I could also see just how much work it would be to generate that data. Yeah, it's collecting a lot of samples, especially with composite sampling where you're collecting lots of samples throughout that time period and then pooling them.

That's quite an involved process. So doing that every day to try and spot those microtrends would be an awful lot of work. Yeah, I think so. So what are the drawbacks of wastewater sampling?

It's not invasive, you don't need consent. You are limited by how much of a drug is there. So if there is, for example, as we've seen with the novel opioids, if there is some there, but it's below your limit of detection, you're never going to see it. So you might think that, oh, novel opioids aren't a problem in

this particular location when in fact they are, it's just there's not enough of it to detect that trend.

So there is quite a big limitation in that regard. That's reasonable. Yeah, and quite a few classes of drug that just don't lend themselves very well to detection in wastewater either. No mention of benzodiazepines in this data set, for example.

I don't know whether they're trickier to detect in wastewater or whether, because a lot of them are like diazepam legitimately prescribed that it doesn't really lend itself very well to like mapping trends. That's fair. Which is when you're looking at purely, I know ketamine has legitimate uses, but cocaine, for example, is used illicitly. I mean, ketamine is used legitimately, probably not anywhere near as commonly as diazepam is used legitimately.

No, you've got a good point. And also, benzos, a lot of them rely on conjugation to end up in the urine anyway. Yeah, that's true. So the amount of free drug is particularly low with benzos.

I think the big drawback is that it only shows you big level data. Yeah. It doesn't tell you anything about an individual. No, it doesn't.

But on a big scale, it is really, really helpful. So what does the future look like for wastewater sampling? I think it's gonna continue to play quite a large role in how we view drug trends overall. It'd be interesting to know if there's any work going on looking at different forms of NPS in wastewater and monitoring those sorts of trends.

But I imagine that's also quite challenging to do, especially if your scope for testing is limited. So alcohol, I don't know whether it's possible to look for alcohol metabolites in wastewater. That would be an interesting trend as well. Yeah, I think it might be, and it would be interesting to see what the trends of that are.

I imagine it's fairly consistent. That's fair, yeah. So you think that it's the sort of thing where you're gonna have a relatively high baseline across all areas and therefore detecting changes might be a little bit less sensitive. Yeah, I imagine that you're gonna have a little spike over a weekend, but...

I would have thought so, yeah. I don't think you're gonna see those sorts of changes, like for example, with festivals or big sporting events that you do with druggies where you do get a massive up spike. I know alcohol consumption will go up, but I think the baseline's probably too high to spot those trends. It's a controversial question.

Is there any impact of these drugs all ending up in the waterway? Oh, I can't imagine it's a good thing. I'm sure I had a talk years ago from someone who was looking at the impact of drugs like cocaine on small creatures in the water. And I believe there was some data that was showing it was having some effects.

Yeah. I mean, we are talking relatively small concentrations, but nonetheless, chronic exposure. Yes. Water-dwelling fish who literally breathe water, constantly exposed to low levels of cocaine.

I'll have to see if I can dig that paper out. Yeah, no, that would be interesting. As well as the differences in water entering a wastewater treatment plant versus the water exiting a wastewater treatment plant and how much that cleanup does get rid of drugs and drug metabolites from the water system in general. That's true, yeah.

So where I was saying about the impact on fish, you're right, that would be post-treatment. How much is being removed by waste treatment is an interesting point, yeah. Well, I hope you've enjoyed listening to us literally talk shit this week. If you found it helpful or interesting, please do consider sharing it.

Giving us a like, giving us a comment. We appreciate all the interactions and a chance to grow the community. Hope you all have an amazing week and we'll see you next time. Bye.