Episode 64: Are Nitazenes Being Missed? Stability in Post-Mortem Toxicology

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Transcript

Hi everyone, welcome back to the Tox Lab. I'm Rebecca. I'm Rob. This week, we're back looking at nitazenes. We're going to be asking the question, are we sure we're picking up all the cases?

So Rebecca, before we look at nitazenes specifically, I want to think in terms of drugs more generally. When we're looking at drugs post-mortem, how do we know we're picking up all the cases? It's tricky, I mean you want to make sure that like your extraction methods can actually extract the drugs you want to look for. You've then got questions on how stable drugs are in samples or in that post-mortem interval.

So there's lots of different factors that come into play. With regards to stability, how do we know whether a drug is stable? I mean, I guess looking at literature for a lot of the common things. So follow-up question, what happens when that drug is pretty much brand new and not been seen before?

Oh, that is a good question. And that's what we're going to be looking at this week. We're actually going to be looking at a story that happened a couple of years back now and the data that it led to has finally been published. And shameless plug, this is a publication that we've been involved in.

Before we jump 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 on there. And for those of you who don't use social media but still want to get in touch with episode suggestions or comments about episodes, you can email us at thetoxlab@gmail.com. So before we talk about today's actual publication, I'm going to open up with the story that led to the development of this paper.

Because it is quite a story. So as I said, we're going to be talking about nitazenes. And we've talked a lot about nitazenes, haven't we? Ultra potent opioids originally developed in the 1950s.

Shelved, never found medical use. Emerged again in 2019 onwards as drugs contaminants in drug supplies. So I want to take us back to 2023, about two and a half years ago. Rishi Sunak was prime minister.

And I guess that means Joe Biden would have been in the White House at this point. Greedy by Tate McRae was on the radio. I'm told. I've got no idea.

No, I've got no idea either. And we were witnessing in toxicology the movement of nitazene drugs from niche outlier cases to something much more mainstream. And this is the story really of two cases that came through our lab. And so the first case was from a known heroin user.

And everything about this case at first glance looked relatively straightforward. She was found deceased with the syringe still in her arm, which is not a common finding, but we do see from time to time. But what was unusual about this case is for whatever reason, they decided to take two sets of toxicology samples, a sample where the individual first arrived at the mortuary and a second sample a week later when the post-mortem happened. Both sets of samples came to our lab.

We analyzed the sample, looked like a classic heroin case, identification of morphine, noscopine, also identified metonitazene and xylazine. And anyway, long story short, I wanted to check a few more things. I wanted to reanalyze the sample because at this point, nitazenes were still fairly new to us. And that initial sample that was collected on day zero, there wasn't enough sample left.

So without really even thinking too hard, I picked up the vial from seven days later and analyzed that. You remember what happened, don't you Rebecca? Yeah, this was when I'd just started properly getting into toxicology after my training had finished. And yeah, it was a really weird set of circumstances.

And so we analyzed that second sample and the metonitazene was no longer there. Now I'm going to be honest, I doubted myself. I thought I had done something wrong. I rechecked everything.

I couldn't go back to that original sample because there was no sample left. I was convinced I had done something rogue. Anyway, maybe a week later, and I was still thinking about this case at this point. We had a second case come in and it was very similar in terms of circumstance.

Another heroin user had sadly passed away. And like the first time, two sets of samples were taken. We analyzed the first sample and sure enough, the pattern was very similar. Consistent with heroin use plus metonitazene, xylazine.

And sure enough, we analyze the later sample. The metonitazene has vanished. So I'm scratching my head at this point. I don't know what's going on.

There was some indication that nitazenes were a little bit unstable. We had a little bit of stability data. I wasn't quite prepared for this. And so I thought about it and I thought about it.

And eventually I spoke to a friend of ours and between us, we hatched a plan to try and test what we had been seeing. And so our paper this week is that study that we conceived. So Rebecca, why don't you dive in, unpack this paper for us. So as we've hinted at, this paper is looking at stability of nitazenes in a rat model.

So 12 male with star rats were given either metonitazene at a concentration of 0.02 milligrams per kilogram or 0.001 milligrams per kilogram. Some were given N-desethyl isotonitazene at concentration of 0.006 milligrams per kilogram. And others were given N-pyrrolidino etonitazene at a concentration of 0.003 milligrams per kilogram. So really, really small doses we're looking at here.

And each nitazene was given to four different rats. The original dose of metonitazene in this, the 0.02 milligrams per kilogram was too high and instantly killed two of the rats. So the lower dose was used for the other two. So when these rats had died, which in four of them was instant, the other eight animals were euthanized.

And then venous blood was collected and stored in both preserved and unpreserved containers. And urine was also collected. The rats were then stored in the fridge for a week to mimic real-world conditions of storage and time frame of bodies being stored before a post-mortem was carried out. And seven days later, blood sampling happened again.

And these again were collected in preserved and unpreserved tubes and stored at minus 80 before being analyzed using iris mass spec following a liquid-liquid extraction. These samples were then also stored in the fridge for a month so we could look at how stable the nittosenes were in that sample ones that had been collected. So we took some rats. They were given nittosenes.

We took samples. We simulated that post-mortem interval and took samples again. And when looking at the results, there was a huge variation in the concentration of each nittosene that was measured

between animals who were given the same dose across both the unpreserved and the preserved samples.

When looking at concentrations of nittosenes between the sample collected on day zero and the sample collected seven days after they'd been in the fridge in preserved containers, there was a reduction in the concentration of the nittosenes with an average of 26.7% of the methanetosene that was measured on day zero remaining on day seven, 36.1% of the end is ethyl isthanetosene from day zero remaining on day seven, and 54% of the end pyridinoetanetosene that was measured on day zero remaining on day seven. And there was also this similar trend detected in the unpreserved samples too.

So considerable loss of nittosenes in that post-mortem interval. Yeah, so we then looked at the samples collected on day seven that had been kept in the fridge for a month as another marker of stability, but within the sample itself. And there was also a reduction in the concentration of nittosenes that measured between that day seven and a month after they'd been in the fridge.

It was a mean average of 20.9% of the methanetosene measured on day seven present a month later, 9.4% of the end is ethyl isthanetosene measured on day seven was present a month later, and 12.8% of the end pyridinoetosene that was measured on day seven was present a month later. So considerable degradation within sample as well. Yeah, we did also look at the urine samples. Nittosenes were detectable in six of the preserved samples and eight of the unpreserved samples, which were analyzed immediately.

They couldn't collect urine on day seven to look at the post-mortem stability of nittosenes in urine. But when the samples were retested a month later, there were similar concentrations of nittosenes being detected in those samples. So up until this point, there was data that showed nittosenes were unstable within samples. We've talked about a paper here before, haven't we, about the instability of nitro-nittosenes and how they can be converted to their amino and acetamido metabolites.

This was the first data, as far as I'm aware, that showed significant loss in that post-mortem interval. Particularly in a relatively short period of time, we were losing considerable amounts of nittosenes. So in this paper as well, a retrospective cohort study was conducted using data from NPSUM, which the national program on substance use mortality, looking at relevant cases from those reported to NPSUM by the 1st of March, 2025. Nittosene cases were identified by searching the post-mortem drug fields by the numerical codes corresponding to nittosene compounds and those from the Birmingham and Solihull Coroniel area.

And this area was chosen because they turn around their inquest conclusions really, really quickly. So the data is most complete from that area. 624 cases were reported to NPSUM where deaths occur between 2019 and 2023 in this Coroniel area. When looking at the presence or absence of nittosenes, the number of non-nittosene deaths in 2023, which were 142 deaths, significantly exceeded the number of deaths that were expected when looking at the trends for 2019 to 2022.

When looking at the presence or absence of morphine slash heroin in these non-nittosene deaths, there was significantly more deaths in 2023 with heroin or morphine being detected than was forecasted from the 2019 to 2022 trend, and it looked to be about a 33% increase. While the number of deaths without nittosenes or heroin or morphine in 2023 did not exceed the expected number, this was towards the upper limit of the model with the actual number being 71 deaths, but the projected number of deaths was expected to be 53 and the upper confidence interval was 73. This paper also covers the profile of deaths following nittosene use.

285 deaths were reported to MPSum by the 1st of March, 2025 that involved nittosenes. The first case was a case of iced nittosene in 2019 where an individual had intentionally purchased this on the dark web, and since then, small clusters of nittosenes had occurred in 2021 with 23 cases and in 2022 with 15 cases, and there was a marked increase in 2023 with 151 cases and the projected number of cases in 2024 was 236. Only 25 of these cases had a purchase intent and nittosenes were the intended purchase item in six of these cases. 12 nittosenes were detected in this data set with seven for the first time being detected in 2023.

Iced nittosene was the most commonly detected nittosene in 2021, covering 78% of these cases, and pyrrolidino s-nittosene in 2022 was the most common covering 60% of the cases, and dazethal iced nittosene was the most popular nittosene in 2023 in 40% of these cases, and protein nittosene was detected most in 2024, covering 77% of the cases in that year. When looking at causes of death, nittosenes were deemed to be the cause of death in 89.8% of cases involving nittosenes, and 97.5% of these cases were deemed unintentional with four being suicides and the remaining three being of undetermined intent.

When looking at the number of nittosenes were detected across these cases, a single nittosene was detected in 87% of cases, two nittosenes were detected in 33 cases, and the most common combination was methanetosene and protein nittosene, and three nittosenes were detected in four cases. Polydrug use was present in all but four cases with cocaine being the most commonly codetected drug in 78.2% of cases, followed by the presence of heroin slash morphine in 72.3% of cases, and xylazine was detected in 33 cases. Naloxone use was also detected in 47 out of the 285 cases.

The majority of deaths in this dataset were from England, which covers 90.2%, and these occurred across the country. 8% of cases came from Wales and 1.7% of cases came from Northern Ireland. And nittosene cases predominantly involved males and a significantly greater proportion of nittosene cases occurred with individuals experiencing homelessness or living in hostels compared to non-nittosene cases. And a history of drug use was significantly higher in nittosene cases compared to non-nittosene cases.

There was a higher prevalence of drug use via injection. So this paper really was a paper in three parts, wasn't it? The stability data, the mathematical modeling, and then the profiling of the cases in which data was known. So let's go back to the stability data first of all.

What are the implications here? Firstly, let's go back to our story, our observation that led to this whole slightly bonkers experiment in the first place. I mean, the implication for me was, okay, I wasn't going mad, I hadn't done anything wrong, which had definitely plagued me for some weeks. But then once you get over that initial feeling of, oh, I was right.

You then start to reflect on the real reality, don't you? That actually, we just don't know how many cases could involve nittosenes. We've got data that shows they're really unstable both within the post-mortem interval and within the sample. We've got, albeit, low case number anecdotal evidence of nittosenes disappearing in real life samples.

How many cases out there could have been nittosene cases whereby there simply wasn't enough or any nittosene to detect by the time the sample was analyzed? I mean, it's so hard to put a number on. I've definitely had the odd case where I'm convinced there was something like a nittosene present that we just couldn't pick up for whatever reason. And it's just one of those unknowns that's just gonna bother me for a long time.

In some ways, it's quite frustrating, isn't it? Because probably isn't just a case of we'll get better equipment. This is the nature of the compounds, how they behave. So I wanna look at the mechanism a little bit because we don't really understand the mechanism, but there are some speculations and some thoughts.

So we know that nittosenes are unstable in blood samples and that's been shown. We think through potentially bacterial conversion to five amino and then acetamido metabolites. What's going on in that short interval between death and sample collection? Well, I guess we know nittosenes are really lipophilic.

So they're probably being redistributed into the fatty tissue like what happens with other drugs, but generally we see drugs that have been taken for a long time like SSRIs, for example, coming out of the fatty tissue during that post-mortem interval. But I feel like it's happening the other way around. You stole my line. That's my hypothesis.

No, you're absolutely right. And that is the hypothesis I've been postulating, that this is like an inverted commas reverse post-mortem redistribution. I can't prove that. We haven't supported that with science as yet.

It really is a speculative hypothesis, but it would explain why, particularly in these cases where individuals have injected a nittosene directly into their bloodstream, that very quickly it seems to be leaving that bloodstream into tissues that aren't the blood. At least that's my hypothesis. So let's look at the mathematical modeling then. That did seem to show that there could have been some cases we were missing.

Yeah, it seemed based on the model there was an increase that wasn't predicted based on the previous year's trends. So there may have been some more cases. Possibly, but what does that mean? No, for sure.

And finally, then let's drill a little bit into the nittosene profile paper. So you talked about that first case. Yeah. And actually that was a case I was involved in.

And that was a really unusual case. Somebody deliberately seeking opioids from the dark web. But since then we've seen that trend, haven't we, towards this being less of a drug that individuals seek and more of a drug that contaminates typically heroin, but sometimes other drugs too. And he's more often than not, not what was intended when individuals took the substance.

Yeah, it's especially scary with nittosenes appearing in counterfeit pharmaceuticals like counterfeit oxycodones or even counterfeit benzos. And that's a trend that does really worry me because those individuals, especially with the benzodiazepines, don't think they're getting an opioid and then do. When nittosenes occur in heroin, the individuals are at least intending to purchase heroin and probably have a degree of opioid tolerance. Yeah.

That isn't the case. Usually if they're deliberately seeking benzodiazepines, they may not have an opioid tolerance. And that general pattern of nittosenes that were described, that really does fit what we saw, didn't it? The move from isotinitazine to endazethal isotinitazine to metonitazene and then to protanitazine later on.

And as we've seen and as we've talked about before, that trend really has continued, hasn't it? We saw then newer nittosenes. And now, possibly, we're looking at a move away from nittosenes towards orphine opioids. And so I'm going to ask you a question now, Rebecca.

How stable are orphine opioids? No idea. Precisely. I'd love to have this experiment repeated with the orphines.

It'd be really interesting to see whether they're one more stable or less stable in the post-mortem to the interval, but also how stable they are in samples. Yeah. I think we've now moved drug classes and suddenly we're back to square one. We're back not knowing again.

Always on the back foot with toxicology. We are indeed. One thing we didn't actually mention at the start was that alongside all of the chaos, the BBC did get involved, didn't they? And they did make a little documentary about this story.

So we'll drop a link as well to that. That is available. It was quite well produced, actually, I thought, and does feature the sound of my voice at points. But it's a different retelling of the same story.

So really, in summary then, I mean, this is, it's going to sound like a really, really bold and arrogant thing to say, but this is the story of science, isn't it? Yeah. A chance observation that led to some awkward inward reflection about whether I had done something wrong, followed by some really solid collaboration with some colleagues, designing a hypothesis, testing that hypothesis, having some results and kind of confirming that initial observation. But also I do, you know, when I reflect on this story a bit, you do have to remember that these were two authentic cases.

These were two people who sadly lost their lives due to nitazenes and they'll never know that legacy that they left behind. So thank you again for listening this week. Do give us a like, give us a follow, share this with your friends, and also check out the paper. We'll put a link down below as we do every week.

Hope you all have an amazing week and we'll see you next time. Bye.