Episode 72: GHB - Forensic Toxicology, Detection and Interpretation (Part 2)

Transcript

Hi everyone, welcome back to the Tox Lab. I'm Rebecca. I'm Rob. This week we're coming back to GHB.

We talked last week about what GHB was, some of the populations of people that use it, and some of the harms and risks that come with its use. And what we're going to be looking at this week is a more forensic angle, how to test for GHB, and also a lot of the challenges that come with testing for GHB, because it's fair to say it is a compound that comes with considerable challenges. Now, please click off this episode and go listen to some of our other content. So very, very briefly, before we dive into this week's papers, GHB is a sedative drug.

It's got a very narrow dose window, so the desirable dose is relatively low, and the toxic dose is not much higher than the desirable dose. And it is capable of rendering people in a state of significant unconsciousness. And that is one of the reasons why GHB is associated with drug-facilitated crime. And so our first paper this week has a look at GHB's role in drug-facilitated crime.

So this first paper aimed to assess the prevalence of GHB in suspected cases of DFC, or drug-facilitated crime, managed in the centre of Paris, restricting sample collection to a maximum 10-hour delay in order to maximise the chances of detection of GHB. And this is a prospective study of three departments likely to receive individuals suspected of having been subjected to drug-facilitated crime, including emergency departments at Cochin and Hotel-Dieu Hospitals, and the Department of Forensic Medicine at Hotel-Dieu Hospital between February 1, 2023 and June 30, 2024.

Patients presenting in one of these three units with suspicion of proactive DFC that occurred less than 10 hours before the presentation and meeting inclusion criteria were included. And the inclusion criteria for this was suspicion of proactive DFC, or drug-facilitated crime, that occurred less than 10 hours before the presentation according to the patient. And this was classed as the victim clearly reporting to the physician the possible administration of a psychoactive substance without their knowledge or consent, and the patient must have been over the age of 18 years.

So proactive drug-facilitated crime is where someone has deliberately given somebody a drug in order to facilitate crime, as opposed to opportunistic drug-facilitated crime, where somebody perhaps is unconscious through drugs of their own use. When patients reported amnesia or neurological symptoms preventing them from giving an exact time of the event, the earliest time of the interval reported was recorded to ensure conservative estimation of the sampling delay for toxicological interpretation.

Three urine samples and two blood samples were collected after a consultation to gather information about the suspected DFC, voluntary psychoactive substance use, and the medical history. Toxicology screening included immunoassay, screen, enzymatic ethanol analysis, and LC high-res MS screening for drugs. 49 patients were included, with 59% of the individuals being from Cochin Hospital Emergency Department. 37 out of the 49 patients were female with a median age of 24.1 years, and female patients were significantly younger than male patients with a median age of 23.48 years versus 26.95 years respectively.

11 patients reported the suspicion of psychoactive substance administration, but were unable to specify if they were also victims of violence due to partial or total amnesia. 12 patients with partial amnesia recalled violence against themselves, and two reported violence against themselves without the presence of amnesia. In nine cases sexual assault without rape occurred in two cases, theft occurred in two cases, and physical violence occurred in one case. 21 patients only suspected the administration of psychoactive substances without their knowledge but didn't suffer from amnesia or violence.

And amnesia is one of the other problems, isn't it? We talked last week a lot about sedation and loss of consciousness, but actually GHB could also cause this amnesia, and it's particularly anterograde amnesia, so difficulty forming new memories. All patients reported at least one symptom during their consultation, with 53.1% reporting total or partial amnesia, and 29.2% reporting other consciousness disturbances. 20.6% reported other neurological symptoms such as dizziness and blurred vision, and a general feeling of malaise was reported in 12.5%.

So 60% of cases occurred at festive venues, which I think means like bars or clubs. 12.5% of cases occurred at the perpetrator's home, one case occurred at the patient's home, and the location was not disclosed in the remaining nine cases. In 91.8% of cases the precise time of the events was known, and the median delay was six hours from event to sampling. For the four remaining cases they could only specify that the event occurred less than 10 hours before the examination.

97.9% of patients reported the consumption of at least one psychoactive substance, and that included alcohol, and 91.8% of patients reported voluntary ethanol consumption. In 20.4% of cases illicit substances were voluntarily consumed prior to the event, with multiple substances being reported in two cases. Cannabis was reported in eight cases, ketamine in one, cocaine in one, MDMA in one, and 3-MMC in one, and no individual reported voluntary GHB consumption.

12.2% of patients reported taking medication with potential psychoactive effects, which included alprazolam, pregabalin, gabapentin, escitalopram, alimemazine, venlafaxine, levetiracetam, and haloperidol. When looking at the toxicology, blood ethanol concentrations were positive in 70.8% of cases with a mean value of 1.32 grams per litre, and urine ethanol was positive in 80.9% of cases with a mean value of 1.64 grams per litre. In both blood and urine, toxicology revealed the presence of one or more voluntarily consumed substances other than ethanol in 13 cases.

THC was the most frequently found in eight cases, followed by prescribed meds in six, cocaine in two, ketamine in one, amphetamine in one, 3-MMC in one, and methadone. In 11 cases, blood and urine revealed the presence of one or more involuntarily consumed psychoactive substances. Six cases were classified as probable proactive DFC with GHB based on statements from the patients and toxicology results where GHB was the only undeclared psychoactive substance detected, and this occurred in one case, and it was found in combination with other non-declared psychoactive substances in five cases. So there's quite a bit to unpack here, isn't there?

They actually did a really good job of trying to only include cases that had had a very, very short time since incident and sample collection. GHB has a real problem, and that is that it is cleared so fast. After GHB use, it might only be detectable for a few hours in the blood, a little bit longer in the urine, but it's still within hours rather than days or weeks, and this poses a massive problem for an investigation of drug-facilitated crime because if GHB is used, it's possible that by the time the person is actually examined and has samples collected, there may not be any GHB left to test.

And so within this data set, there were a few cases, weren't there, where GHB was detected? So in the cases where GHB was detected, the mean age was 40.3 years and the majority of individuals were male. Five had reported voluntary ethanol consumption and two reported voluntary substance use,

one being cocaine and the other being 3-MMC and one also reported taking pregabalin. Two individuals did report a context of chemsex, but GHB was not knowingly or intentionally taken in these cases.

Five individuals reported amnesia, which was described as total in one with suspected sexual violence and partial amnesia in four where two reported rape, one reported associated sexual violence and one reported theft. One patient did not have any amnesia and didn't report any violence. When looking at the blood samples, three individuals showed a mean GHB concentration of 46 milligrams per litre while the other three had a GHB concentration of less than 5 milligrams per litre. GHB was detectable in all six urine samples and showed a mean GHB concentration of 576.3 milligrams per litre.

In five other cases, probable proactive DFC was suspected based on statements and toxicology that did not involve GHB but involved another psychoactive substance. These included cocaine, ketamine, zopiclone, THC, quetiapine, oxazepam, ethylamphetamine, 3-CMC and alprazolam. All of these individuals had reported voluntarily taking psychoactive substances in addition to this with three experiencing partial amnesia and three reporting rape.

17 individuals reported violence or suspected violence and there was no reported psychoactive substance present but they had all consumed ethanol or a psychoactive substance voluntarily so these were thought to be opportunistic cases and 15 out of 17 reported partial or total amnesia. So I think what's really crucial here is that GHB was present in a few although actually the range of substances that was implicated was quite wide wasn't it? It wasn't just GHB, it wasn't even just benzodiazepines. Yeah.

I know this study really set out to try and look for GHB and that was why it had such narrow inclusion criteria and you could see in some of those cases that were positive in the urine the blood had cleared. Yeah. And that was hours later and that is one of the problems with detecting GHB in these contexts or in any context it has such a short half-life that actually detecting it can be a real problem particularly if there's a delay between a crime occurring and it actually being reported and investigated and we know that's not uncommon.

So another issue with GHB is that it is colourless and odorless so it can quite easily be slipped into people's beverages in the context of spiking. One of the things I do want to talk about, you mentioned it briefly actually, but there was a lot of alcohol use in that subset wasn't there and alcohol and GHB do interact in a couple of different ways don't they? So both GHB and alcohol act through GABA receptors with alcohol primarily acting through GABA-A and GHB can act on GABA-B receptors but these both lead to central nervous system depressions so using both of them will cause accumulative CNS depressant effects.

But when you mix GHB and alcohol you can also increase the half-life of GHB and there are a couple of possible mechanisms for how this occurs. So GHB is metabolized to succinic semi-aldehyde by the P450 enzyme SIP2E1 and this is an oxidative process and it's catalyzed by GHB dehydrogenase. Part of the metabolism of ethanol also uses the same P450 enzyme so there could be some competition for this preventing GHB from being broken down as quickly. In addition to this in the metabolism of ethanol using alcohol dehydrogenase and aldehyde dehydrogenase can increase the NADH to NAD plus ratio which can then inhibit the metabolism of GHB.

So that is one of the areas where forensic detection of GHB can be really problematic in this drug-facilitated crime and actually we're not going to dive too hard into it but this is true probably in impaired driving as well. I don't know how often GHB is implicated in impaired driving but you would have the same issue this very short detection window and it could make investigating crimes like this really challenging.

But another area where GHB is particularly hard to detect is in the context of post-mortem toxicology and we've already said haven't we, GHB can be potentially fatally toxic and some people really do skirt that line between pleasurable use and death quite closely. We're going to take a look now at the detection of GHB post-mortem because it has a few additional challenges and one of the other challenges in the post-mortem context is that GHB actually can be formed post-mortem. So the mechanism behind post-mortem GHB formation isn't entirely understood so this is possibly due to enzymatic conversion of GABA, succinic acid and putrazine.

Bacterial production of succinic acid from glucose can lead to further GHB production and complications further. So when looking at GHB production in blood post-mortem a cutoff of between 30 and 50 milligrams police has been suggested to exclude post-mortem production but there have been cases of post-mortem GHB production being greater than this 50 milligram police a cutoff.

Post-mortem GHB is also producing other matrices such as urine but this occurs to a lesser extent so you can look at the ratio of blood to urine and a low ratio between urine and blood can indicate post-mortem formation whereas in cases of actual GHB ingestion urine concentrations tend to be approximately 10 times that of blood although this is dependent on time between ingestion and death for this equilibrium to be reached so most people use a cutoff of about two.

So in this study they wanted to investigate the proportion of cases where GHB was formed post-mortem and how this linked to the GHB concentration and they also wanted to look at the ratios of GHB in urine and blood and they also looked at the effect of the post-mortem interval and the degree of co-formation of ethanol in cases of post-mortem formation of GHB. So in the study they looked at toxicology data from 2000 to 2021 at the department of forensic sciences at Oslo university hospital and this covered more than 39 000 individual cases.

All cases with a valid positive GHB result in blood and urine were included and when looking at data regarding the co-formation of ethanol cases were included between 2011 and 2021 because during this time they were measuring ethyl glucuronide and ethyl sulfate. And so detection of ethyl glucuronide or ethyl sulfate shows you that alcohol had been consumed prior to death whereas if you didn't detect those any alcohol that was detected could be put down to being post-mortem formation. When detecting GHB prior to 2010 they were using a method using GCFID and then moved on to a HPLC tandem mass spec method.

When looking at whether a case was positive or negative for GHB they had a blood cutoff of 31.2 milligrams per liter and a urine cutoff of 10.4 milligrams per liter and they defined post-mortem formation of GHB as a ratio of urine to blood below 2. They also looked at the ICD-10 code T41.2 which covers deaths caused by intake of GHB and excluded these cases from the post-mortem formation group. So in other words if a case was determined to be a GHB case they excluded it so we didn't skew the data because of exogenous GHB consumption.

Yep and they also defined post-mortem formation of ethanol as a positive ethanol combined with a negative ethyl sulfate result. GHB was detected above the cutoff values in blood and urine in 525 cases and the median age was 57 years and 84% of individuals from AIL. The median concentration of GHB in blood was 45.8 milligrams per liter with a range from 31.6 milligrams per liter to 1477.6 milligrams per liter and in urine the median concentration was 27.4 milligrams per liter and this range from 10.6 milligrams per liter to 7518 milligrams per liter and the median post-mortem interval was seven days. So this is all cases isn't it?

So this will include cases that have also got GHB consumption involved. Yep in 66 cases the urine to blood ratio was above two which indicated GHB intake in the median concentration of blood was 174 milligrams per liter and a median concentration in urine was 914.5 milligrams per liter but in 10 of

these cases there was a blood concentration below 52.1 milligrams per liter. In 87% of the cases post-mortem formation was expected due to the ratio of urine to blood being below two but in 12 of these cases GHB intake was expected as they were excluded from the data.

So that's interesting so 12 cases in this set there was reason to think GHB had been consumed but that ratio actually didn't hold. Yep in these cases the median blood concentration of GHB was 268.4 milligrams per liter with a range of 64.8 milligrams per liter to 1477.8 milligrams per liter. And that's a massive range isn't it? Huge amounts ranging from relatively low concentrations to you know grams a liter.

Among the 447 cases with post-mortem formation of GHB the median concentration of GHB in blood was 42.5 milligrams per liter and the median concentration in urine was 24.9 milligrams per liter. The post-mortem interval in this group was significantly longer compared to cases where GHB was ingested and this was seven days versus three days respectively. There was also a statistically significant relationship between the concentration of GHB in post-mortem formation cases and the post-mortem interval. And that's not surprising is it?

The longer the post-mortem interval was the higher the post-mortem GHB formation. The most frequent main cause of death in the post-mortem formation cases was diseases of the circulatory system followed by ill-defined and unknown causes of mortality and poisonings. Nearly a third of the 447 cases showed concentrations of GHB of 52.1 milligrams per liter or greater while 19 cases were above 156.2 milligrams per liter. Six of these 19 cases had a diagnosis of intoxication of other drugs would be used without mentioning GHB and GHB co-ingestion could not be excluded in these cases.

The other 13 cases with a raised GHB died of a cause not related to drug ingestion. At blood GHB concentrations between 31.2 milligrams per liter and 41.5 milligrams per liter, 99% of the cases were results of post-mortem formation. Whereas cases with a blood GHB concentration between 104.1 milligrams per liter to 156 milligrams per liter, 63% of them were post-mortem formation. And when looking at concentrations above 156.2 milligrams per liter, 29% of these cases involved post-mortem formation of GHB.

So you can see that by moving that cutoff higher you can exclude some of the post-mortem formations but even at a cutoff of 150 you've still got a decent chunk of cases there where it's post-mortem. And the median urine to blood ratio was 0.52 for post-mortem formation of GHB. Just to put some of this into context, in anti-mortem samples, so in non-post-mortem samples, significant sedation can be somewhere between 100 to 200 milligrams per liter. So there's a huge overlap between these post-mortem concentrations and what would be significantly active in a person who was alive.

So when looking at cases of post-mortem GHB formation and the post-mortem co-formation of ethanol there were 430 cases to analyze and 27% of these had post-mortem co-formation of ethanol. And the median ethanol concentration at these was 0.26 grams per kilogram which is equivalent to 28 milligrams per 100 millilitres. And there was no significant relationship between the concentration of GHB formed post-mortem and the concentration of ethanol that was formed post-mortem. Where both were formed post-mortem the median post-mortem interval was 11.5 days and in case where ethanol was not co-formed to the GHB the median post-mortem interval was six days.

And this is interesting as well it seems there's no relationship between production of ethanol versus the production of GHB. And there's possibly a few reasons for that isn't there? Post-mortem ethanol formation is unpredictable. Yeah.

And whilst there's a few patterns longer post-mortem interval tends to increase it and trauma can increase it and so on. There's no real rhyme or reason to it. Some cases there's post-mortem ethanol

there, sometimes there isn't. It seems to be to do with the presence of different bacteria in the decomposition process.

And it possibly makes sense that the species of bacteria that ferment glucose to ethanol are not the same species that convert substrates to GHB. And ultimately the conclusion from this paper that a cutoff for post-mortem formation of GHB should not be set higher than 30 milligrams per liter. And as we've said that's a relatively low cutoff isn't it? And this just shows the complexities.

Many many cases significant potentially significant concentrations of GHB are produced post-mortem. And this can become a real forensic challenge can't it? This study is really good because it shows you the prevalence and by looking at ratios in the urine you can kind of have an idea. We don't always get urine post-mortem.

Not every case can urine be collected. And this then starts to show some of the scale of the problem. If you've got a post-mortem blood sample with a GHB of 150 and for whatever reason a pathologist couldn't get a urine sample, is that post-mortem formation? Is that potentially significant toxic GHB consumption?

It does just show how you need to look at the case as a whole and look at the history and the circumstances around it as well as the toxicology to be able to kind of pull these elements together to aid interpretation. You can't just rely on a nice clean cutoff of like urine to blood ratio or a cutoff in blood to work out whether something was post-mortem formation or was potentially significant.

And it would be really interesting I talked about at the beginning of this paper that GHB is formed in other matrices like urine but also it's formed in vitreous humor to a lesser extent than blood and it'd be really good to see how blood versus vitreous humor GHB ratios play a role in helping to determine whether it's post-mortem formation or GHB intake in cases where you can't get urine. One other point we haven't touched on actually is the analytical challenges that come with GHB and GHB is a bit of a pain of a molecule. It's a small tiny little molecule.

It's quite water soluble and there is another endogenous compound called beta hydroxybutyrate which I'm sure we've talked about before.

We measure it as a marker of ketoacidosis in post-mortem cases but it can be present at reasonable concentrations in the living as well and it's isobaric to GHB and so you need a chromatography method that can separate GHB and BHB and GHB doesn't tend to separate from BHB very well on a lot of the chromatography conditions that we will do our general screening on and so quite often labs need a separate method to do GHB analysis and so it's sometimes the case that many labs will only do GHB testing if it's indicated and I think this probably means we are missing a significant number of cases.

We said in the last episode didn't we about how sometimes GHB is used in chemsex circles and in men who have sex with men but actually we're starting to see its use outside of those circles and I do worry actually that we are missing cases because that additional screening step isn't being carried out particularly if you've got other drugs there that may be potentially contributory it's possible that that extra step isn't being done.

So I think this really shows some of the challenges that GHB testing brings doesn't it both the short half life the post-mortem formation and the fact that it may require additional testing on top of other broader tests.

So really in summary then GHB is very much a thing and whilst its prevalence in total is perhaps relatively low it does seem to be increasing in its harms and possibly being used in circles that we may not have previously associated it with and it also poses a lot of challenges to toxicologists both

analytically and biologically the fact that it's produced post-mortem at concentrations that could be potentially significant really do hinder investigations to a degree don't they? Yeah. Well I hope you found listening to this insightful and helpful in some way. Do give us a like, give us a follow, share it with your friends.

Thank you very much to everyone who has tuned into this we really do appreciate you being here. Hope you all have an amazing week and we'll see you next time. Bye!