Episode 78: Strange Things in Strange Places - Poison Dart Frogs, Navalny and Epibatidine

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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 some iconic poisonous creatures and the toxins that they produce, but we're going to go on a little bit of a journey to get there first because we've actually got a strange case to unpack, and this case is unique because it features something strange in a very unusual place. So Rebecca, strange things in unusual places.

Come across many of those? I'm sure I have, my mind goes completely blank and I can't think of any examples. For me one of the surprising things in a strange place was the stolen World War II painting which was identified in the background of some photos when someone was trying to sell a house. Now you said that that does ring a bell.

I think for me one of the strangest things in an unexpected place is the Philpott Lane mice statue. So it's this tiny little statue in London of two mice fighting over a piece of cheese and it's really really really high up on this building and that one always makes me smile when I see it. Because it's just something you don't expect. Yeah.

You look up and you see a statue of mice fighting over cheese. Yeah it's great. Fair enough. One actually caught my eye recently, biology related.

My daughter's really into marine biology so every so often we catch up on the marine biology news and this one was a bit awkward to try and explain to her because scientists have actually observed fish hiding in the anuses of manta ray. Oh wow. And that is definitely a surprising thing in a strange place. For sure yeah.

But the reason we're talking about surprising things in strange places by far one of the strangest things in surprising places for me this year has been the presence of poison which is normally found in poison dart frogs in South America actually being found in a penal colony in the Arctic. Now we are of course talking about the case of Alexei Navalny.

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 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. And we do now finally have our own website. We are at the Tox Lab dot co dot uk do go and check out the shiny new website and actually also maybe give it a bit of a stress test click around if you find any bugs let us know.

I don't think we've got too many but it would be really good to have feedback on that. The hope is we'll be able to start putting transcripts of the episode up there as well. We've got a few on there already. I just need to work through the backlog another way for people to sort of engage in the content really.

I also want to give a shout out to someone on Spotify who suggested that we cover this in an episode so a big thank you to HSMCC36 for suggesting this. So Rebecca why don't you intro us to this case then. So in February 2024 Alexei Navalny a prominent Russian opposition figure died suddenly in a

prison colony in the Arctic. Russian authorities said it was due to natural causes but questions were raised almost immediately and Navalny had already survived a poisoning in 2020 when international labs confirmed he'd been exposed to Novichok.

But he was taken to hospital and he did recover from this. Now we covered Novichok back in episode 54 a little while ago. Two years after his death a joint statement from the UK, Sweden, France, Germany and the Netherlands revealed something unexpected. Analysis of samples had in their words conclusively confirmed the presence of epibatidine which is a toxin associated with poison dart frogs.

And the joint statement claimed they believe that the Russian state were involved but as of this point Russia denies all involvement and still stands by the natural cause of death argument. And this is a really odd finding isn't it? Yeah it's quite unexpected. A poison that's found in frogs in South America somehow ending up in an Arctic penal colony.

There's a bit I'd like to unpack a little here because this joint statement was issued. I don't think there's been any evidence any data beyond the statement. Also my understanding is that it was tested in a sample of blood that was smuggled out of the penal colony. So we've not got an established chain of evidence.

No. This would if this were a criminal court be torn apart in cross-examination. I'm not saying that it wasn't there. I'm not saying the scientists are wrong.

I'm just saying that there are still quite a lot of unanswered questions about how strong we can take this finding to be. But if true it's weird. Poison dart frog poison should not be anywhere near the Arctic Circle. And in terms of the case this is really all we know isn't it?

Yeah there's really not a lot of confirmed data out there on this. I believe there is some witness evidence that said before he died he did show some signs of illness. Yes yeah I believe so. And those symptoms could be associated with poison dart frog poisoning but it's all a little bit woolly and inconclusive.

So why don't we dive a bit into what this compound is and talk a bit about poison dart frogs. So toxins found in the skin of certain species of dendrobated frogs have been used to poison the grooved tips of blow darts for hunting in western Colombia which is kind of where poison dart frogs get their name from. And research into these compounds began in the mid 60s. And poison dart frogs are iconic aren't they?

Yeah. They're absolutely beautifully coloured. We might talk a bit about the colouration later actually. I'm going to tell you some lore about me.

Oh okay. When I was younger I was terrified of poison dart frogs. Were you? The idea that these tiny little creatures that could jump really high really fast and if they landed on your skin could kill you was really scary.

I don't know how old I was probably like 13 or something. Yeah. No I was younger than that. But they really were for me as a child quite scary and I was quite fascinated with them.

Oh see I really liked seeing them in the reptile and amphibian house at the zoo. But anything beyond the frogs, when you start getting into the snake bit, it's a no-go for me. That's interesting. I was absolutely fine with the snakes.

It was the poison frogs that used to freak me out. But as you say they're called dart frogs. I used to think because they were fast moving but actually it's because they were used to poison darts. And I'm told the way they would extract the poison from these darts.

Fun fact or not fun depending on your perspective is they'd roast the frogs over a fire. Oh I think that's a fun fact. It sounds cruel. It does sound cruel but it's a fun fact.

So these compounds were found to be esteroidal alkaloids and were given the collective name batrachotoxins and these were shown to be potent and specific activators of sodium channels. Other dendrobatid frogs from Panama had bicyclic alkaloids including pomillotoxins which are relatively toxic and are potent myotonic agents which have modulatory effects on sodium channels. So you've used lots of words. Let's take this back a notch.

So poison dart frogs aren't one thing. No. I said about how you get all these beautiful different colors. They're all different species and what is interesting is they produce different poisons depending on where they're found which species they are.

Yeah and it's important to mention that frogs don't synthesize the toxic alkaloids that are present on their skin rather they sequester them into their skin glands from dietary sources. So we think although I don't think it's actually conclusive that it's things like termites and beetles that actually produce the compounds and the frogs have evolved a way to sequester that and excrete it from their skin. Yeah in a similar way to tetrodotoxins and pufferfish. Pufferfish and sea stars.

We talked about that a few episodes ago didn't we. Yeah. And this actually brings us on to another point. My fear of dart frogs when I'd visit the reptile house may have been completely irrational because dart frogs in captivity actually lose their poison.

This is true yeah. Because they're not exposed to the source of poison in the first place in their diet. It's moving on to epibatidine. This was isolated in 1974 by Myers and Daly from the dendrobated frog.

Epidobates tricolour from two sites in southwestern Ecuador. An epibatidine is fascinating. Of all of the poisons found in poison dart frogs it's perhaps for me the most interesting and it's the most interesting for a very nerdy reason and that is it's an organic compound that actually contains a chlorine atom. That is really nerdy.

Hear me out. If you're doing high res screening of a blood sample and you see something with a chlorine in, in my experience that's a drug of some kind. Yeah. But actually this is an organic compound containing a chlorine atom occurring in nature and that is really really rare.

Apparently it's a lot more common in sea creatures. Okay. So we think the source of the epibatidine ultimately before termites and ants or whatever it is that the frog is being exposed to is likely some bacterial source. Okay.

But either way absolutely fascinating chemistry for a naturally occurring organic compound to have a chlorine atom in it. And this compound also caught scientists' attention in that it was believed it could be a painkiller right or it was shown that it could be a painkiller. Yeah it was found to be 200 times more potent than morphine in experiments in mice and interestingly it wasn't antagonised by naloxone. So it was recognised to be a painkiller.

I'd love to know what made them test it. Yeah I'm not sure. But at the time a lot of the good painkillers were all opioids and interestingly they thought this could have been an opioid at that point but it wasn't antagonised by naloxone. So it's later found that this compound is a nicotinic acetylcholine receptor agonist.

So it binds to nicotinic acetylcholine receptors. They are a class of receptors that are sensitive to the neurotransmitter acetylcholine. They're found throughout the body often on muscles but also quite a lot in the spine and central nervous system. And that's actually I think the rationale for why it has an analgesic effect because it interrupts pain pathways within the spine.

They're called nicotinic acetylcholine receptors because nicotine also binds to them and activates them. So epibatidine is essentially like nicotine on steroids. So epibatidine binds to the alpha-4 beta-2 subtype of these receptors, leading to its analgesic effects, and because it has a higher affinity for the receptor than nicotine, for example, it can lead to overstimulation of the receptor causing symptoms such as salivation, hypertension and tachycardia. Eventually there is desensitisation of the receptor leading to muscle paralysis and if this occurs in the respiratory muscles this can be fatal.

This is the rationale for dart frog poisoning leading to essentially death. Yeah. It paralyzes the muscles associated with breathing and these symptoms actually you've described hypersalivation, hypertension and tachycardia. We've discussed these before.

This is quite characteristic of quite a few classes of poisons including cholinesterase inhibitors and that's why there's a bit of overlap. So cholinesterase inhibitors stop the breakdown of acetylcholine and acetylcholine is what naturally triggers these receptors. Epibatidine hijacks these receptors, activates them really really hard for a really long period of time and leads to this prolonged desensitisation. So companies have attempted to produce structural analogues of epibatidine that have the analgesic effects without the huge risk of paralysis that comes with these to market them as an alternative painkiller.

So one of these was tebanicline which was produced by Abbott Labs. It also had the name ABT-594 and this showed potent analgesic activity and was less likely to cause paralysis. However its development was stopped after its phase two clinical trials where it had an unacceptable incidence of GI side effects. And there is clearly a market for non-opioid painkillers, particularly ones like this where it had a really really really strong analgesic potential.

The problem was it came with this risk of paralysis and ultimately death. It would be interesting to see where these compounds go actually because in an ideal world they could be really helpful right? Yeah I know there were lots that were in the early stages of development that then got dropped but it'd be interesting to see whether they picked up now whether we could make them work or make an alternative work. And there's not really any treatment for this poisoning is there other than supportive care.

The main mode of death is through respiratory paralysis so artificial ventilation will keep somebody alive. It's possible Atropine might have an effect maybe. Atropine works on the other class of acetylcholine receptors so possibly not going to have such an effect on nicotinic acetylcholine receptor agonists. Like so many rare toxins there isn't a real one-size-fits-all antidote it's just intensive care and supportive care that really helps people survive it.

So fascinating compound Epibatidine. I want to have a look a little bit at the frogs themselves because why aren't the frogs poisoned? So that is a really interesting point. So other organisms that have developed resistance to poisons such as tetrodotoxin and cardiac glycosides do this by altering amino acids at the binding site to the relevant receptors so that these compounds can't bind.

And this mechanism works because the receptors that are affected by tetrodotoxin and cardiac glycosides are not ligand-gated receptors but in this case you've got a nicotinic acetylcholine receptor that Epibatidine is binding to so this mechanism doesn't quite work. So Epibatidine resistance requires decreasing sensitivity to Epibatidine whilst maintaining the sensitivity to endogenous acetylcholine and this is challenging as Epibatidine and acetylcholine will interact with many of the same amino acids at the receptor.

So there has been some work on this and four sites on the beta 2 subunit of the alpha 4 beta 2 nicotinic acetylcholine receptor have unique amino acid replacements which are close to the

Epibatidine binding site. The problem with this is it leads to a decrease in sensitivity to acetylcholine.

So frogs have also developed further amino acid replacements leading to a decrease in sensitivity to Epibatidine without affecting the sensitivity to acetylcholine so you've got these two different types of mutations in amino acid replacements to stop poison dart frogs being affected by Epibatidine and what's really interesting is three different types of poison dart frogs that produce this toxin have evolved these mechanisms independently from each other so it does show how important it is for poison dart frog survival to have these mutations in place.

I think from an evolutionary biology perspective this is absolutely fascinating isn't it so if I've understood this right they have a mutation that means they are more resistant to Epibatidine but in order for them to survive the resistance to Epibatidine because that will also have a knock-on effect on their endogenous acetylcholine they have another mutation that counteracts that. Yeah it's really really cool. How that has evolved I don't understand I'm honest I mean I'm obviously not an evolutionary biologist but that seems really counterintuitive to have required two mutations in order to see this phenotype.

I think the whole family of poison dart frogs are fascinating because as we said there's actually multiple different species and they all sequester different poisons I guess they must have some similarity in how they handle some of these things although perhaps not what you've just described is so specific to Epibatidine and also they only seem to be found in South America. Yeah. So there must be some common ancestor surely. I think there probably is but I don't know I'm not the frog expert.

If anyone is a frog expert listening do get in touch and let us know. I mean I think as toxicologists these creatures are absolutely fascinating unlikely to ever come up in our day-to-day casework I suspect although strange things and surprising places. Never say never. Never say never.

If you've got an unknown and it's got a halogen it's probably a benzodiazepine or something like that but you never know it might be a frog poison. I think it's a really interesting area and it kind of brings up what other compounds are out there in really weird niche species hidden in rainforests that might potentially have a therapeutic use. That's really true. So many of our therapeutic compounds have originated in nature and you're absolutely right you know what else is out there.

I mean we've just focused really hard on Epibatidine but as we said there's lots of different poison dart frog poisons. I'm not sure I suspect people will have done some of the work to see whether there might be therapeutic potential for some of these. The other I think really really cool thing about poison dart frogs now I'm over my fear is just how brightly coloured they are. Yeah they are really really impressive to look at and this is that classic.

You see it a lot in insects don't you where dangerous insects are brightly coloured. You don't see it in more complex animals often but actually these frogs are an exception aren't they where they're brightly coloured to warn off predators. And another thing about their appearance that I found quite surprising is how tiny they are. Like some of them are really really small like a centimetre big when they're really grown.

Yeah really little because you see a nice close-up photo someone's taken with a macro lens and you assume it's what we think of as frogs in the UK like frog size and no they're tiny. Yeah you're right. So taking it back to the case then well clearly if there really was Epibatidine found in Alexei Navalny that would be a strange thing in a surprising place. As we said currently this is denied unconfirmed in terms of the fact that actual analytical evidence hasn't emerged.

But nonetheless it does leave you wondering. Well I hope you've enjoyed listening to us this week. Thank you again for suggesting that we have a look at this fascinating subject. I've certainly learned a

lot looking at these iconic animals and they're iconic poisons.

And if you have enjoyed as always do give us a like, do give us a share, do go check out our new website. We'd also appreciate that. Hope you'll have an amazing week and we'll see you next time. Bye!