

Mirjam de Bruin-Hoegée, from the University of Amsterdam, tells Gwyn Winfield about her research on detecting types of fentanyl in the body

I sing the body fentanyl

Fentanyl really ticks all the boxes. It's driving the narcotics epidemic that is ravaging the US, with over 67,000 people killed in 2021¹. It's used as a tool in murders² and robbery³ and also for assassinations⁴. Such is its potency that it fits into the pharmaceutical based agents part of the spectrum of fourth generation agents, and has been considered by some a weapon of mass destruction⁵. Consequently the drug is a focus for law enforcement, the military and paramedics.

Although the drug's offensive potential was known for a long time, even before its use in the Moscow Theatre Siege of 2002, it only burst into

public consciousness as a threat when its role as a recreational narcotic become common. Initially there were concerns that it could penetrate the skin and it was not unusual for panic attacks to be seen as symptom of potential fentanyl exposure⁶. Now, an improvement in training and understanding (including by our own CBax – *CBRNe World* 2022-02) has largely mitigated that fear, but the potential for the misuse of this agent by bad actors remains - it's the only agent with this level of potency that can be summoned into existence with a credit card.

The prevalence of fentanyl in crime scenes has brought together a wide variety of detection techniques, from basic colorimetric BTNX strips, all the way through to field portable mass spectrometry, such as the Griffin or MX908. Organisations like the

Clandestine Lab Enforcement Team (CLET - see *CBRNe World* 2022-03) are now looking to take data from the more sophisticated identifiers and start to build up a portfolio of chemical fingerprints from within the makeup of the drug. Chemical forensics can tell you how the drug was made, what precursors it was made with, what brand of precursors were used, and whether there were telltale failures in the production that can link it to a certain batch. All this information can then be compiled and attached to a production facility and potential individual for prosecution.

Once the agent is in the body, however, the biggest signifiers for death are the trappings of drug use around the body, or alternatively powder still on the clothes from the aerosol release. Now, however, a technique is being

Mirjam de Bruin-Hoegée research will allow scientists to understand which method was used to create fentanyl from in vivo samples
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pioneered by Mirjam de Bruin-Hoegée from the University of Amsterdam and guest researcher at the Netherlands Organisation for Applied Scientific Research (TNO), but currently seconded to the US National Institute of Standards and Technology (NIST). This offers the chance to do *in vivo* the same kind of chemical forensics that can be done *in vitro* - ie using samples from within the body, rather than the agent itself.

Using gas chromatography with a flame ionisation detector (GC-FID) and liquid chromatography orbitrap mass spectrometry she was able to look at the metabolites within liver microsomes and find out, for example, what method was used to synthesise the fentanyl (Gupta or Siegfried). This is after the

drug has been metabolised. So if there was a clandestine introduction of fentanyl to the individual, in the air for example, hours later the laboratory could not only confirm a synthetic opioid as the cause of death, but reveal which fentanyl derivative it was, how it was created and potentially any telltale signifiers in the sample⁷.

The important piece to get initially from this research is the mission space. Fentanyl analogues can be extremely potent in small doses, and mixed with other narcotics such as heroin, they could give off a really tiny 'signal' amongst a lot of white noise. With such a low concentration we might only find a small amount of metabolites. This would be different if we compare it to a

crowd of healthy people (such as soldiers) who were subjected to a large scale release of pure fentanyl, giving them a massive spike in their liver metabolites.

What sort of application was de Bruin-Hoegée aiming at? "It can be applied to both areas. In our lab we were originally looking at weapons of mass destruction for the military, but fentanyl is a very wide problem, so this technique can be used by police for one overdose victim as well. When a sample is less pure, there could be more interesting information in it, as the impurities can be related to the production. Alternately if another compound is the main element of the sample, I would rather focus on the



The more active agent there is in a sample the more information you can get from it ©J. Shanley

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In theory her research could be used for production methods of other drugs and CWA ©J. Shanley

impurities of that compound than fentanyl, because it would be easier.”

De Bruin-Hoegée’s research came out of conventional work that TNO was doing on detecting traditional chemical warfare agents in biomedical samples. [As an interesting aside, we heard about this work from Dr Daan Noort, the head of the Organisation for the Prohibition of Chemical Weapons’ new chem lab. Ed.] de Bruin-Hoegée was just ‘lucky’ that fentanyl has far wider applications in the security field than agents like VX, for example.

The two examples given above, injection vs inhalation, follow different routes into the body, and might therefore have different biomarkers. Inhalation, for example, will have more of an initial reaction with the lungs than the liver. Yet the liver is the body’s ‘garbage recycler,’ so was there something that made the liver the best target for getting the chemical information of how the agent was created, for example? “We did *in vitro* studies with the liver microsomes, but it would be possible, in theory, to pick

other tissues, such as lung microsomes if that was the main mechanism of action. For example, an *in vitro* study to the more potent analogue carfentanil has identified lung metabolites as well⁸. We could then do the same experiments with incubation of lung microsomes using fentanyl and test for these compounds. In the case of the liver, fentanyl metabolises to another compound, but if it remained stable then it would still be interesting because the impurities might also remain stable and it might be easier to detect in biomedical samples.

“As to whether it is injected or inhaled, it then either goes into the lungs or the bloodstream. What you mostly see is the time. Fentanyl converts into norfentanyl, and if it was injected we’d see the same set of compounds, but other studies indicate that the ratio could be different.”

The concentration level that de Bruin-Hoegée worked on makes her fairly confident that it could be picked up in individual drug overdoses, that it wouldn’t need to be a massive spike.

Obviously the stronger/more concentrated the sample of fentanyl the more information on the impurities can be extracted, but she was still happy that they would be able to get useful information on the process and precursors even within a medical dose.

The ability to get any information on the material will provide vital knowledge. Even learning the method used to make it provides vital information to law enforcement, but being able to provide data on the type of fentanyl, how it’s made and what it’s cut with, all from metabolites might be a game changer. De Bruin-Hoegée thought it could happen, but maybe not yet... “In the *in vitro* study we demonstrated that it is possible, but you need to verify it with real whole blood samples from victims and then have a very large database and dataset to compare with, and that is currently lacking. There’s plenty of data available, police and other labs have a lot of measurements and data, but they still have to analyse it and compare it with others. So the data is there, but

we'd need to make comparisons and see the trends before it could be used in court. In terms of the additives, baby powder, for example, would need a separate study. Another study would be all the fillers on a database. For our part, we focused on the fentanyl and mainly on the precursors used, so if different precursors were used, we could distinguish between them. If there was a batch with the same precursors but made on different days, it might be harder to distinguish between them but small changes in concentration of the precursors could affect the profile, and some of these impurities would be visible in the blood. It might be hard, but theoretically it should be possible. The same would be true for other drugs."

De Bruin-Hoegée did admit that if there were two drugs in one sample, then similar impurities could confuse some of the biomarker data. She stated that in one case they had acetal-fentanyl as an impurity, but it's also a drug on its own. That would be an example of something that could be 'pure' drug, or a mixture of the impurities of two drugs.

Currently the study has all been lab based, and as in much of CBRN there is a big difference between the lab and the 'battlefield'. There's also the impact of death on the body and what this would mean for the biomarkers. If you are looking for metabolites then these might be paused on death, or broken down and altered at time of death. How long could the body be stored in a mortuary, for example, before all signal is lost? Was there an optimum time, a sweet spot for collecting these samples?

De Bruin-Hoegée suggested that this would have to be the next stage of the research. "We should extend the study to real case victims. After death there are very different studies about how far fentanyl is metabolised. It seems it really depends on the person, and

whether it is inhaled or injected. It's going to be difficult to provide a general sweet spot."

The next step of the project is likely to be looking at a wider variety of pure samples, and relating that to the liver microsomes, to bring the two bits of research together. "Linking this data to the data of intact drug samples will be the next stage. I've seen monitoring about what kind of drugs appear and how they are made, and that's easier to do in pure batches rather than biomedical samples. Once we know that, we can relate the biomedical samples to these batches, but I think that can be concurrent research. In the future it would be good if we can monitor more of what we measure in the labs, because even though the data is there we don't use it because we're only focused on what compound it is, rather than what profile do I see?" Collecting this data will be important for future research.

"I hope to still be involved in it, as I am interested in this, and have been

discussing the idea with colleagues from the University of Amsterdam. They are going to look to all the old spectra from police labs to see whether they can find trends in the profile of the drugs by monitoring street samples, so maybe this can later be extended to biomedical samples."

It's a sad truth that there will always be more fentanyl samples to look at than chemical warfare agents. Tracking down the chemical fingerprint that will implicate particular facilities will provide the kind of high value information that will become more commonly required in criminal investigations. The labs' experience in processing this will eventually return to the chemical warfare space, so when the inevitable happens and another dictator uses chemicals against his own people, or enemy combatants, the skills, techniques and people will be there to ensure that there is a successful prosecution in The Hague.



A library of cutting agents could also be included to assist n prosecution ©J. Shanley

¹ <https://injuryfacts.nsc.org/home-and-community/safety-topics/drugoverdoses/data-details/#:-:text=The%20fentanyl%20category%20of%20opioids,the%2053%2C480%20total%20in%202020.>

² <https://www.youtube.com/watch?v=dqJ-Mrp4SwY>

³ <https://www.cbsnews.com/newyork/news/nypd-makes-arrest-in-robbery-and-drugging-spree-in-manhattan/>

⁴ <https://www.timesofisrael.com/begging-royal-mercy-how-israel-recovered-from-the-botched-mashaal-hit-25-years-ago/>

⁵ <https://inkstickmedia.com/fentanyl-is-a-dangerous-drug-not-a-weapon-of-war/>

⁶ <https://www.npr.org/2023/05/16/1175726650/fentanyl-police-overdose-misinformation>

⁷ <https://www.sciencedirect.com/science/article/pii/S2468170921000266>

⁸ <https://link.springer.com/article/10.1007/s11419-019-00521-x>