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Distinguishing Intake of New Synthetic Cannabinoids ADB-PINACA and 5F-ADB-PINACA with Human Hepatocyte Metabolites and High-Resolution Mass Spectrometry

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Guest: Dr. Marilyn Huestis is the Chief of the Chemistry and Drug Metabolism Branch of the National Institute on Drug Abuse.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

Synthetic cannabinoids are novel psychoactive substances which are designed to stimulate the endocannabinoid system and mimic the effects of the main psychoactive constituent of cannabis. Originally developed as research tools, the consumption of synthetic cannabinoids, that is, so-called "legal highs" has gained popularity in the past decade.

Use of these substances has produced dangerous behaviors, severe intoxications, and fatalities, leading many countries to schedule them as illicit drugs. However, the market for synthetic cannabinoids is dynamic with new versions constantly replacing those that are newly controlled, leaving legislators struggling to keep up. Research to identify the metabolism of these new substances and to identify potential markers of their use is needed.

An original research article appearing in the May 2017 issue of *Clinical Chemistry* provides this insight for two of the newest and most potent synthetic cannabinoids. We're joined by the article's senior author, Dr. Marilyn Huestis. Dr. Huestis is the Chief of the Chemistry and Drug Metabolism Branch of the National Institute on Drug Abuse.

Dr. Huestis, welcome back. Do we expect the emergence of novel psychoactive substances to continue into the foreseeable future?

Dr. Marilyn Huestis: We certainly do, and it's a frightening future because we originally started with compounds that had been published, their legitimate synthesis had been published, and it began with synthetic cannabinoids. Of course, those had been synthesized by legitimate chemists to use as research tools to study the neurobiology of the human body and mind. And so these compounds that were much more potent than the original Delta-9-Tetrahydrocannabinol, or THC, that is present in marijuana or cannabis, they were trying to create

compounds that were more potent than the typical ones in cannabis.

They did, and then one after another after another began to be produced and available by the market. According to the DEA, the Drug Enforcement Agency, the profit margin on these synthesized drugs is so large and the potential for the clandestine laboratories that are primarily in China, but now other countries as well, it's so great that the potential for being caught is so low that this form of drug marketing is definitely here to stay.

Bob Barrett: Why is it so important to determine the human urinary metabolites of novel psychoactive substances?

Dr. Marilyn Huestis: Well, because these compounds are so potent, they are given in low doses, or at least they should be given in low doses, but this is why we have so many overdoses and now deaths related to these compounds. Because as they are clandestine labs, there is no quality control involved and there's no control to ensure that you have an even distribution of drug. So, we have terrible overdoses and deaths.

The problem is that with these low doses, unless you get a blood sample very rapidly or an oral fluid or saliva sample very rapidly, the parent drug is non-detectible. And so what people generally have to do then, is turn to urine testing and of course urine testing is always one of the best matrices to use because we know that the drugs are present in higher concentrations, and you can detect the presence or the intake of those drugs for a longer period of time.

But, with the novel psychoactive substances, we have no idea how these drugs are metabolized. Many of these drugs that originally, the actual synthesis had been published, now the clandestine labs are going off of that and creating new compounds all the time. The reason is to hope to avoid the scheduling of these drugs, in the United States and many other countries around the world, so they will change the molecule enough that they hope that it will not be covered under the new laws, and so we have these brand new compounds that we don't know anything about their pharmacodynamics, how they affect the body.

We clearly don't know how the body metabolizes these drugs, the pharmacokinetics of these drugs, and so we don't even know what to be looking for in urine samples. So, that's why it's absolutely key that we do the kind of studies that we're talking about today in order to rapidly publish what the human metabolites are, so that laboratories around the world can be taking the spectra and the molecular weights that we provide and being able to use

those to search compounds. So when there is an outbreak of a particular synthetic cannabinoid, in this case, they can at least look for the metabolites to be able to warn public health officials, first responders, emergency rooms, of what is out there.

In many cases, it's enabled public health offices to ramp up their response to the release of these new drugs. So, it's absolutely critical that we do that. We had a beautiful relationship with the Drug Enforcement Agency that enabled us to do this, because you can imagine that with the rapidity of which these drugs are coming on the market and according to the EMCDDA, which is the European monitoring organization that has been really at the forefront of all these emerging drugs, there have been more than 650 brand new novel psychoactive substances from many different drug categories that have been released since 2008.

So, you can see what a problem this is for toxicologists around the world. We have definitely been busy handling just the amphetamines and the cannabinoids and cocaine and opiates, which is our major epidemic that's going now, and a few other drugs. Now, we've had 650 new compounds released in less than 10 years. You know, it's a phenomenal problem.

Bob Barrett: Doctor, what instrumentation and biological and software tools did you use to determine the optimum metabolite urinary markers of THJ-018 and THJ-2201?

Dr. Marilyn Huestis: Well, for biological tools, we buy human hepatocytes. They have to be used while they're still alive and viable. They are taken from individuals who die and whose liver cells are still functional, and they are frozen at a very low temperature to keep them viable. So, when we first receive these cells, we have to check to make sure that they are still working well, and they are very expensive.

In general, it's about $1,000 for a very small vial that we can only do somewhere between three and five compounds at one time, and then we use those cells, and we also use human liver microsomes. Human liver microsomes, we use to determine how rapidly that the hepatocytes will use that compound, because unless we know how fast the drug is metabolized, we can't set up our experiments appropriately.

Then we use tandem liquid chromatography mass spectrometry in order to identify how rapidly the drugs disappear and are metabolized.

And then we have to use high resolution mass spectrometry in order to determine the different fragments and metabolites that are produced. It's like taking a jigsaw

puzzle of maybe 2,500 pieces, throwing it up in the air, letting them fall to the ground, and trying to put a puzzle together without a picture. So, it's very intense work to try to determine which are the metabolites from the many fragments that are formed.

So, while it only takes us a few days to do the actual work with the human liver microsomes and human hepatocytes, it takes us a month or more to determine what those specific metabolites are. Another key part of the tools that we use for this research, is we use software that can predict the metabolism of drugs. You can't rely fully on that but it can give you candidate compounds, but as we have done this work, we've been able to see when software may fail us and not predict specific metabolic products. And then we also use a lot of software that helps us interrogate the data and determine what the metabolites are. This technique is very dependent on both the biological software as well as instrumentation tools to be able to predict the optimal metabolite urinary markers.

Bob Barrett: What are the advantages and disadvantages in determining synthetic cannabinoids urinary metabolites using human hepatocytes and/or human liver microsomes?

Marilyn Huestis: Well, human liver microsomes is the tool that most people use, because human liver microsomes are much less expensive and also because it doesn't require specialized laboratory areas. You have to use a special biotype 2 laboratory in order to be able to do this work, but the reason that we've chosen, and have learned to use human hepatocytes is because human liver microsomes may produced a large number of potential metabolites. But because the microsomes are not an intact cell, they're just a portion of the cell, they don't tell you which are going to be the most prominent or prevalent metabolites that you expect to see, where human hepatocytes are an intact liver cell and therefore when they produce a metabolite, it's taken into consideration factors like having to pass through plasma membranes and get into human hepatocytes and it takes into consideration the normal environment of that human hepatocyte to produce the metabolite.

We have done both and we've learned that human hepatocytes give you the top metabolites that you expect to see rather than a huge spectrum of analytes and you don't know which ones are the most important. By the time we finish our work, we're able to say, "These are the most likely prevalent metabolites. Put these three metabolites into your mass spectrometer and look for these analytes." If you do human liver microsomes, you have to look for maybe 20, 25, 30 metabolites because you're not sure which one is

going to be the most important. So, it's really been very useful to do this.

One thing that I really want to get across to the audience is that initially the novel psychoactive substance problem begins with synthetic cannabinoids, like the two that we're talking about today. However, now we have synthetic cathinones, they were the second group to come. Unfortunately, we have to say now there are synthetic opioids which have fueled our current opioid epidemic. We have synthetic benzodiazepines and the list just goes on and on. You realize with the synthetic opioids that they can be a thousand fold more potent than say heroin and also they have put out many compounds that they say they're heroin or they're oxycodone, but they're actually these much more potent compounds, so the unsuspecting user may die. And in this country, in the United States, in 2015, we had more people die of opioid overdoses than we did from car crashes. So, you can see what a frightening future we have with these novel psychoactive substances.

Bob Barrett: Well finally, doctor, should the data be published if no human urine sample is available to confirm the metabolite predictions?

Marilyn Huestis: Well, we always try to get authentic urines samples from the new drugs that we are looking at. The problem is these are brand new and the Drug Enforcement Agency, when they start to see a lot of seizures in the United States, they will take some of those compounds. They'll purify them and they will send that to us before they are scheduled.

We then rapidly did the work and published the work as quickly as we could so that laboratories around the world had tools to try to address these new drugs. The problem is that then it made it very difficult for us to find human urine samples. We have collaborations around the world where they would send us urine samples from overdoses, sometimes even when they didn't know what drug was taken. And then we would interrogate those compounds and try to verify in authentic human samples what metabolites were found, and verify our work.

About, I would say, more than half of the drugs that we've done so far, we were successful in being able to get these urinary metabolites. Unfortunately, for the drugs that we're talking about here, we did not have any human urine samples available. We still think it's very valuable to publish the data because at least individuals can use this to screen and look for these drugs; otherwise, they don't know what to look for.

I think it is still well worthwhile to publish the data. However, it brings up another major problem in this area. One, is the fact that we need standards from commercial reference standards makers, and we need not only the parent drugs which are much easier for these reference standard makers to do, but we need the metabolites. And so that's a second reason why it's so key to publish the data because then the standard manufactures, they know what three, say, compounds they should focus on. If they don't know that, they're very reluctant to go through the expense of producing 20 or 25 different metabolites with the hope that one or two of those might be key metabolites.

Bob Barrett: Dr. Marilyn Huestis is the Chief of the Chemistry and Drug Metabolism branch of the National Institute on Drug Abuse. She has been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.