Clinical Chemistry 62:1
4–5 (2016)

Editorials

Putting Designer Drugs Back in Pandora's Box: Analytical Challenges and Metabolite Identification

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History is full of stories of scientific discoveries being used in unexpected ways. Just think about how the technology of microwaves, developed to be used in radar technology, revolutionized the lunch break, or how the antiarrhythmic drug Viagra improved the potency of middle-aged men. The story of designer drugs is similar, although with another outcome. Many claim it started in the 1980s with work to develop agonists of the CB1 and CB2 receptors for patients with chronic pain; these receptors are activated by tetrahydrocannabinol (THC),[3] the active ingredient in marijuana.

The 20 years leading to 2008 resulted in many pain-relieving compounds that had been evaluated and discarded. However, some of these compounds started to reappear among recreational drug users, as first observed by the group of professor Volker Auwärter in Germany *(1)*. This group of compounds, known as spice or synthetic cannabinoids, marked the emergence of new psychoactive substances (NPSs).

NPSs, also known as designer or Internet drugs, include several groups of drugs including the synthetic cannabinoids mentioned above, designer benzodiazepines, synthetic cathinones, and the *N*-2-methoxy-benzyl (NBOme) drugs. They have all been designed and manufactured by humans, some most likely replicated from the scientific literature *(2)*, and others from small modifications of other illicit or prescription drugs.

There has been an explosion of new NPSs. In 2014 alone, 101 novel NPSs were reported by the European Monitoring Center for Drugs and Drugs of Abuse *(3)*. The sheer number of new substances has overwhelmed forensic and clinical toxicology laboratories and has placed huge demands on method development and validation. Likewise, this phenomenon has put a tremendous strain on legislative bodies responsible for scheduling these new substances. It does seem that the scientific community, in its effort to develop new drugs, unintentionally opened Pandora's box just enough to let the NPSs out.

We cannot turn back the clock and undo this problem. NPSs are here to stay, and we must deal with the consequences. Substances identified after police seizures and newly synthesized compounds should be incorporated into the screening methods of the toxicological laboratory, preferably before any positive cases have been observed. The use of high-resolution mass spectrometry with data-dependent acquisition has greatly reduced the need for revalidation and optimization when adding new substances to the screening methods.

In this issue of *Clinical Chemistry,* Diao et al. *(4)* report on an effort to characterize the metabolism of 2 synthetic cannabinoids, THJ-018 and its 5-fluoro analog THJ-2201, after human hepatocyte incubation. One of the main reasons users choose NPSs is that many of them are invisible in the drug screening process. An important first step is therefore to develop suitable analytical methods to measure these drugs and suitable strategies to rapidly deal with novel NPSs that emerge.

The above approach is succeeding for some groups of NPSs but not for others, such as the NBOmes, the designer benzodiazepines, and the synthetic cannabinoids. The reason is that most drug testing is conducted using urine. Compared with blood, urine is less invasive and easy to obtain, and some drugs have longer detection windows in urine than in blood *(5)*. However, for the NPSs indicated above, none of the parent compounds can be detected in urine, and metabolites must be used as markers of drug intake. Because the metabolism of novel NPSs is rarely known, and few, if any, positive cases are available, in vitro studies of NPS metabolism have become an important part of NPS method development.

It is important to identify markers that are both abundant in the urine and specific to the drug in question. Most groups use human liver microsomes because the system is cheap, simple, and robust, whereas a few groups use cryopreserved hepatocytes, a more complete metabolic system capable of more reactions. The difficulty of using an in vitro approach is that you see only the liver metabolism, and it is difficult to predict which are the major metabolites in urine, assuming that they are even formed using the in vitro model system.

Another approach is using in vivo data from either authentic case samples or animal models such as mice or

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Received October 6, 2015; accepted October 15, 2015.
Previously published online at DOI: 10.1373/clinchem.2015.248096

[3] Nonstandard abbreviations: THC, tetrahydrocannabinol; NPS, new psychoactive substance; NBOme, *N*-2-methoxy-benzyl.

rats. Metabolism of animals is different from that of humans, and therefore the major metabolites in mouse or rat urine might not be the same as in human urine. This issue is avoided by identifying the metabolites in authentic cases, but the analyst then must deal with several confounding factors. First, normally nothing is known about the amount or time of drug intake or if there has been repeated intake. Second is the interindividual variability in drug metabolism. The major metabolites in 1 case might differ considerably from those observed in another. Therefore, it is difficult to draw general conclusions from 1 or 2 authentic urine samples. Third, not only are the synthetic cannabinoids a group of very similar compounds, but also the substances are often impure and mixed with each other. This means that even if you have a number of authentic urine samples, it is possible that the samples were collected from individuals who smoked the same mixture, meaning that the metabolite observed in some or all of the samples could have originated from an impurity or other substance in the mixture. To properly assess the metabolism of a synthetic cannabinoid, different approaches need to be combined and the results must be interpreted carefully.

The work of Diao et al. *(4)* shows how this process can be carried out successfully and sets the standard for future studies. The authors combine the use of human liver microsomes and hepatocytes to make the most of in vitro experiments. They predict relevant parameters such as clearance of the drug, as well as identification of the major metabolites that are suitable targets in drug screening. The presented results should be confirmed in a larger sample set, but because NPSs spread quickly around the globe, the publication of this work is very timely.

The methodology used by Diao et al. *(4)* represents an important step in understanding and controlling these drugs, but given the scale of the NPS problem, we need to do more. The scientific community should work closely with the manufacturers of reference materials to provide relevant compounds (parent drugs and metabolites) for screening in urine. We also need to collaborate with decision makers in the pharmaceutical industry to further streamline the process of scheduling novel NPSs. These 2 efforts should make NPSs somewhat less interesting to producers and users of illegal drugs and thus make the problem of NPSs a little bit smaller.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

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