

## Designer Drugs Keep Evolving Novel Psychoactive Substances Challenge Laboratories and Laws

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**D**esigner drugs, research chemicals, novel psychoactive substances (NPS), and legal highs are all terms used to describe drugs designed to skirt legal controls. Although the trend toward using these drugs picked up steam in the new millennium, the search for new recreational substances—either through repurposing pharmaceutical research or modifying existing drugs of abuse—is not new.

The second International Opium Convention, effective in 1928, strictly limited the manufacture and distribution of morphine and heroin (1). In response, chemists began synthesizing esters of morphine such as benzoylmorphine and acetylpropionylmorphine, which have effects similar to those of heroin but were considered legal alternatives at the time.

### LSD and the Beginnings of Designer Drugs

Dr. Albert Hofmann, a chemist at Sandoz Laboratories in Basel, Switzerland, was investigating potential medical uses of ergot alkaloid derivatives when he synthesized lysergic acid diethylamide (LSD) in 1938. He discovered its psychedelic properties when he accidentally ingested it. In 1947, Sandoz Laboratories introduced it as a medication to treat psychiatric disorders (2).

LSD became a popular drug for psychiatric research and recreational use. In 1968, the U.S. Drug Enforcement Administration (DEA) classified it as a controlled substance, and shortly thereafter, the synthesis of ALD-25 or 1-acetyl-LSD led to the first prosecution based on a drug analog.

In 1965, Dr. Alexander Shulgin obtained a DEA license and began synthesizing chemicals in a backyard laboratory, thus launching the modern era of designer drugs. Shulgin developed a protocol for evaluating new drugs that included initial testing in animals

before human experiments began. The protocol involved increasing doses and using a rating scale to determine an effective dose. The subjects of these experiments were friends and acquaintances of Shulgin and his wife.

The Shulgins published narratives of these experiences in two books, *PIHKAL: A Chemical Love Story* (1991) and *TIHKAL: The Continuation* (1997), with PIHKAL standing for “phenethylamines I have known and loved” and TIHKAL standing for “tryptamines I have known and loved.” These two books are often considered to be “cookbooks” by manufacturers looking to introduce these drugs to a new generation of users.

### Cannabinoid Studies

While Shulgin was evaluating stimulant and hallucinogenic drugs in California, at Clemson University, Dr. John W. Huffman was studying the cannabinoid receptor system to understand its role in diseases such as multiple sclerosis. Huffman’s team synthesized more than 400 compounds that have varying degrees of binding affinity and activity at cannabinoid receptor 1 (CB<sub>1</sub>) and cannabinoid receptor 2 (CB<sub>2</sub>).

CB<sub>1</sub> is the receptor responsible for the nervous system effects perceived as a “high” caused by delta-9-tetrahydrocannabinol (THC), the primary active component of marijuana. CB<sub>2</sub> is primarily a peripheral receptor involved in immune system modulation. Huffman was searching for compounds with affinity for and activity at the CB<sub>2</sub> receptor, but more

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## Designer Drugs

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than two decades later, experimental drug users rediscovered his work and the potential of CB<sub>1</sub> receptor agonists.

### Tracking Psychoactive Substances

In 2007, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) expanded the scope of its Early Warning System to include not only new synthetic drugs but also new psychoactive substances (3). Every year since then, the system has reported a steady increase in the number and scope of new compounds. In 2009, it officially noted 24 new compounds for the first time; in 2015, there were 100 (3,4).

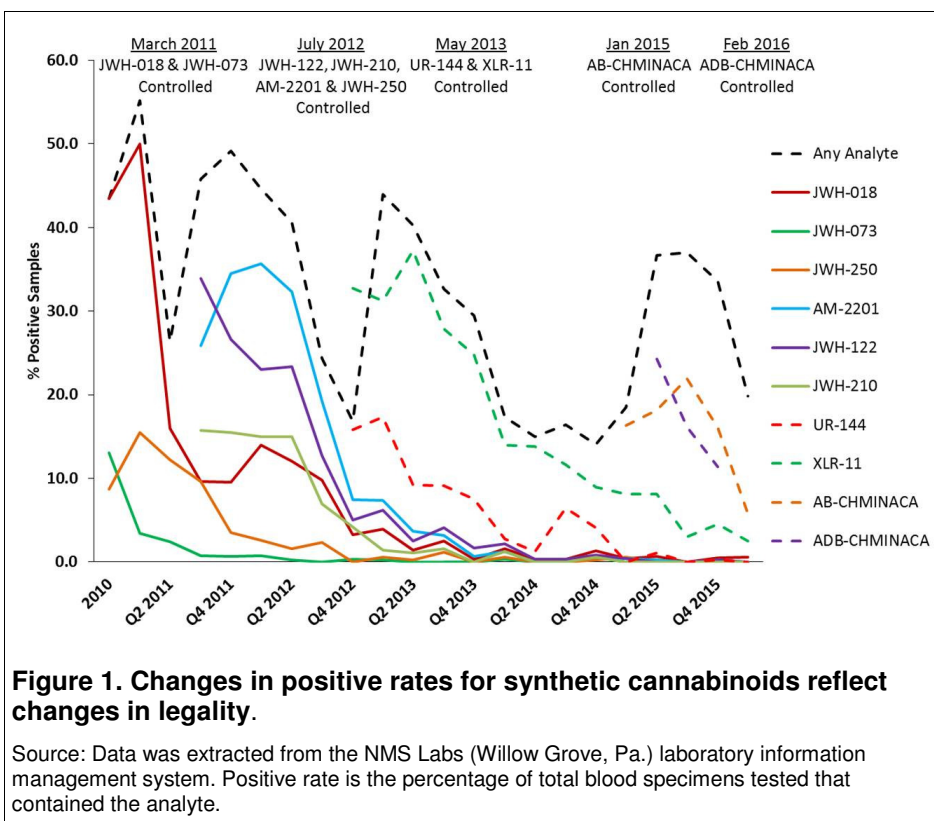
The EMCDDA now monitors more than 560 new compounds, 380 of which have been added to the list in the past five years. It reports a sevenfold increase in the total number of seizures of NPS from 2008 to 2013 (4). Not only has the number of monitored compounds increased, the types are also changing. In addition to synthetic cannabinoids, designer opioids and benzodiazepines have gained popularity in recent years.

### Synthetic Cannabinoids

The first synthetic cannabinoid detected in the U.S. was one known as HU-210, which U.S. Customs and Border Protection found in packets of an “herbal incense” product called “spice” in late 2008. HU-210 was already a controlled substance in the U.S., but shortly thereafter an uncontrolled drug, JWH-018, was detected in similar products. This compound was one of the 400 substances synthesized by Huffman’s research group.

Since early 2009, the components of these herbal incenses have changed rapidly. Typically, as soon as the DEA adds a substance to the list of controlled substances, chemists make slight modifications to the structure to create a compound that is not listed. Figure 1 shows the ebb and flow of compounds detected in routine testing by a reference laboratory compared with their legal status.

To aid in the comparison and identification of synthetic cannabinoid structures, the EMCDDA developed an online interactive tool that defines the basic structure of a synthetic cannabinoid as containing a ring, a link, a core, and a tail (Figure 2). However, this model does not accurately describe the newest



**Figure 1. Changes in positive rates for synthetic cannabinoids reflect changes in legality.**

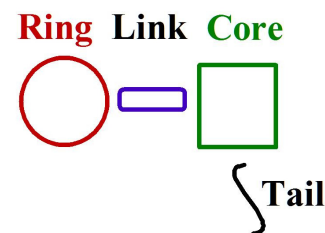
Source: Data was extracted from the NMS Labs (Willow Grove, Pa.) laboratory information management system. Positive rate is the percentage of total blood specimens tested that contained the analyte.

compounds, which no longer contain a ring structure.

According to a thorough review of the pharmacology and toxicology of synthetic cannabinoids published in 2014, recreational synthetic cannabinoids primarily target the CB<sub>1</sub> receptor, but many also have effects at the CB<sub>2</sub> receptor (5). It is possible that CB<sub>2</sub> receptor activity modulates some of the undesirable effects seen with the pure CB<sub>1</sub> receptor agonists.

### Mouse Tetrad Test of Effects

The most common *in vivo* test for cannabinoid effects is the mouse tetrad test, which evaluates the effect of a compound on several different mouse behaviors. This series of tests determines the dose of drug required to change specific behaviors by 50% (ED<sub>50</sub>). By comparing the ED<sub>50</sub> values of drugs, one can estimate their potencies. For example, the average ED<sub>50</sub> for THC is 2 μmol/kg and the ED<sub>50</sub> for JWH-018 is 0.7 μmol/kg (5). Therefore, JWH-018 is approximately three times more potent than THC. Unfortunately, the mouse tetrad has not been performed on the



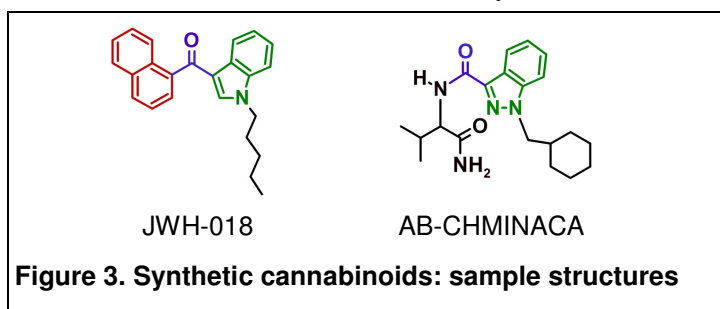
**Figure 2. General structure of synthetic cannabinoids**

Source: <http://emcdda.europa.eu/topics/pods/synthetic-cannabinoids#panel2>

vast majority of synthetic cannabinoids, so this information is available for only a handful of them.

Figure 3 provides examples of structures, and Table 1 gives chemical names and potency data.

Adverse effects associated with synthetic can-



**Figure 3. Synthetic cannabinoids: sample structures**

**Table 1. Selected synthetic cannabinoids: common name, chemical name, and potency\***

JWH-018 (1-pentyl-1H-indol-3-yl)-1-naphthalenyl-methanone	2.9 <sup>a</sup>
JWH-250 1-(1-pentyl-1H-indol-3-yl)-2-(2-methoxyphenyl)-ethanone	NA
AM-2201 [1-(5-fluoropentyl)-1H-indol-3-yl]-1-naphthalenyl-methanone	NA
JWH-122 (4-methyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone	NA
UR-144 (1-pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)-methanone	6.5 <sup>b</sup>
XLR-11 (1-(5-fluoropentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone	6.3 <sup>b</sup>
AB-CHMINACA N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide	26 <sup>c</sup>
ADB-CHMINACA (MAB-CHMINACA) N-[1-(aminocarbonyl)-2,2-dimethylpropyl]-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide	NA
5F-AMB N-[[1-(5-fluoropentyl)-1H-indazol-3-yl]carbonyl]-L-valine, methyl ester	NA
5F-ADB N-[[1-(5-fluoropentyl)-1H-indazol-3-yl]carbonyl]-3-methyl-D-valine, methyl ester	NA

\*Potency is compared with delta-9-THC as determined by mouse tetrad studies. NA = No published potency data is available.

<sup>a</sup>Gurney SM, Scott KS, Kacinko SL, et al. Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs. *Forensic Sci Rev* 2014;26:53–78.

<sup>b</sup>Wiley JL, Marusich JA, Lefever TW, et al. Cannabinoids in disguise: Δ9-tetrahydrocannabinol-like effects of tetramethylcyclopropyl ketone indoles. *Neuropharmacology* 2013;75:145–154.

<sup>c</sup>Wiley JL, Marusich JA, Lefever TW, et al. AB-CHMINACA, AB-PINACA, and FUBIMINA: affinity and potency of novel synthetic cannabinoids in producing 9-tetrahydrocannabinol-like effects in mice. *J Pharmacol Exp Ther* 2015;354:328–39.

nabinoids include tachycardia, hypertension, agitation, hallucinations, nausea, vomiting, tremors, seizures, anxiety, and paranoia. The types and degrees of effects vary significantly from compound to compound. The Centers for Disease Control and Prevention (CDC) issued five “Notes from the Field” related to adverse events of synthetic cannabinoids between November 2013 and October 2015.

In February 2013, the CDC reported on acute kidney injury associated with XLR-11 use in 16 patients (6). The next two reports, in late 2013, were related to severe illness associated with ADBICA and AB-PINACA. These drugs, which were in herbal incense products, led to 147 patients being seen in emergency departments, with 16 admitted to intensive care units, but no reported deaths (7,8). The June 2015 report related a general increase in adverse events from synthetic cannabinoid use but did not identify any specific compounds (9). The most recent report came in response to a cluster of hospitalizations resulting from the use of ADB-CHMINACA (10). Nine deaths were reported out of 119 patients who received medical care.

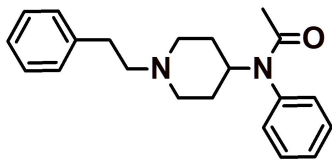
## Opioids

Novel opioid compounds have a longer history than synthetic cannabinoids, but there was a long period of inactivity with respect to new compounds. That hiatus ended in 2013 when acetyl fentanyl burst onto the recreational drug scene. Several other opioid-related compounds followed in late 2015. These compounds primarily stem from substances synthesized but abandoned at some point by pharmaceutical companies conducting pain management drug development research. They are chemically diverse, and most are not similar in structure to opiates, although they generally target the μ-opioid receptor. Activation of this receptor causes the analgesia, sedation, and euphoria desired by users. Adverse effects include itching, nausea, constipation, and decreased respiration. Users develop the tolerance to both the desired and undesired effects of opioids that requires larger and larger doses.

The development of tolerance and the availability of street drugs of unknown composition are a dangerous combination. An additional danger is that drug users and drug manufacturers add some of these compounds as adulterants in commonly abused opioids such as heroin.

The potency of these compounds varies greatly. Individuals who believe they are using heroin may take their usual dose, but the presence of another, more potent, opioid could make the dose deadly. Another concern is the increased toxicity of opioids when combined with benzodiazepines. Taken alone,

prescription benzodiazepines rarely result in death, but their combination with opioids can have a synergistic effect on respiratory depression.



**Figure 4. Novel opioid compounds sample structure: acetyl fentanyl**

**Table 2. Selected novel opioid compounds: common name, chemical name, and potency\***

Acetyl fentanyl N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-acetamide	15.7 <sup>a</sup>
Furanyl fentanyl N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-2-furancarboxamide	NA
Butyryl fentanyl N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-butanamide	7.0 <sup>b</sup>
<i>para</i> -Fluorofentanyl N-(4-fluorophenyl)-N-[1-(2-phenylethyl)-4-piperidinyl]-propanamide	15.7 <sup>a</sup>
3-Methyl-fentanyl N-[3-methyl-1-(2-phenylethyl)-4-piperidinyl]-N-phenyl-propanamide	48.5-569 <sup>b</sup>
AH-7921 3,4-dichloro-N-[[1-(dimethylamino)cyclohexyl]methyl]-benzamide	0.77 <sup>c</sup>
U47700 <i>trans</i> -3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methyl-benzamide	NA
MT-45 1-cyclohexyl-4-(1,2-diphenylethyl)-piperazine, dihydrochloride	0.77 <sup>d</sup>
W-15 4-chloro-N-[1-(2-phenylethyl)-2-piperidinylidene]-benzenesulfonamide	1000 <sup>d</sup>
W-18 4-chloro-N-[1-[2-(4-nitrophenyl)ethyl]-2-piperidinylidene]-benzenesulfonamide	10,000 <sup>d</sup>

\* Analgesic potency is compared with morphine as determined by acid writhing tests. NA = No published potency data is available.

<sup>a</sup> U.S. Drug Enforcement Administration Office of Diversion, Control Drug & Chemical Evaluation Section. Acetyl fentanyl (2015). [http://www.deadiversion.usdoj.gov/drug\\_chem\\_info/acetylfentanyl.pdf](http://www.deadiversion.usdoj.gov/drug_chem_info/acetylfentanyl.pdf) (Accessed June 2016).

<sup>b</sup> Higashikawa Y, Suzuki S. Studies on 1-(2-phenethyl)-4-(N-propionylanilino)piperidine (fentanyl) and its related compounds. VI. Structure-analgesic activity relationship for fentanyl, methyl-substituted fentanyls and other analogues. *Forensic Toxicol* 2008;26:1–5.

<sup>c</sup> Technical Report on 3,4-dichloro-N-[[1-(dimethylamino)cyclohexyl]methyl] benzamide (AH-7921). EMCDDA, Lisbon, April 2014.

<sup>d</sup> Nakamura H, Shimizu M. Comparative study of 1-cyclohexyl-4-(1,2-diphenylethyl)-piperazine and its enantiomorphs on analgesic and other pharmacological activities in experimental animals. *Arch Int Pharmacodyn Ther* 1976;221:105–21.

## Opioid Structure and Activity

Figure 4 provides a sample structure, and Table 2 provides chemical names and potency data for selected novel opioid compounds identified in routine casework at a large commercial reference laboratory. In general, these compounds have been evaluated by comparing their analgesic activity to that of morphine or fentanyl by using an acid writhing test (11). This test involves exposing a mouse to an irritant with and without the study compound on board to determine the dose required to reduce writhing by 50%. This is a test of analgesia—no conclusions about the mechanism of analgesia can be reached nor can the results be used to classify a compound as an opioid.

The compounds in Table 2 represent a small sample of opioids with abuse potential that have or may make an appearance in the NPS marketplace. The patents that describe the synthesis of many of these compounds include many other substances that might be candidates for illicit use. For example, the patent that includes W-15 and W-18 has information on 32 compounds, 14 of which have analgesic potency greater than that of morphine (12). (This discussion includes the “W” compounds in the opioid section because media reports routinely refer to them that way. However, no information has been published on their binding to opioid receptors, and the mechanism of their analgesic action is not known.)

Overdose with novel opioids is assumed to be similar to that observed with any other  $\mu$ -opioid receptor agonist, and—like overdoses with heroin or prescription opiates such as oxycodone or oxymorphone—can be treated with naloxone. Naloxone acts as an antagonist at the  $\mu$ -opioid receptor with such a strong binding affinity that it replaces opioid agonists. This receptor blockade quickly reverses the potentially life-threatening central nervous system (CNS) depression caused by opioids. Because naloxone is extremely safe, several states have made it available over the counter, and many first responders are equipped with it to treat opioid overdose. The need for this antidote is demonstrated by the increasing number of novel opioids found postmortem.

## Benzodiazepines

Benzodiazepines are among the most commonly prescribed psychotropic drugs in the U.S. There are 14 benzodiazepines available by prescription to treat a variety of physical and psychological conditions. Compounds such as midazolam and triazolam have half-lives of two to three hours and are often used to induce anesthesia or as sleep medication. Alprazolam, lorazepam, and clonazepam are used to treat anxiety, panic disorders, and seizures, with effects lasting for

six to 10 hours. Chlordiazepoxide (a muscle relaxant) and diazepam (an anti-anxiolytic) are two examples of long-acting benzodiazepines, with half-lives exceeding 24 hours.

Benzodiazepines target the gamma-aminobutyric acid A (GABA<sub>A</sub>) receptor. This receptor has multiple binding sites, which may explain the differences in degree and type of response observed from different benzodiazepines. All benzodiazepines have sedating effects, which makes them a target for abuse, but they are generally safe.

### Novel Benzodiazepines

Novel benzodiazepines are often referred to as “research benzos” by drug users because they include medications available in other countries but not legal in the U.S. and compounds that cannot be prescribed legally in any country.

Phenazepam and etizolam were the first two benzodiazepines to appear in the illicit drug market in the U.S. Between 2008 and 2013, there were 284 reports of phenazepam in 31 states (13). Etizolam appeared in 2012 with a total of 140 reports up to June 2014, according to the DEA (14). Other compounds that have been reported include bromazepam, flubromazepam, flubromazepam, delorazepam, diclazepam, and clonazepam. Figure 5 contains sample structures of novel benzodiazepines; Table 3 includes chemical names and places where they are legally marketed.

Some signs and symptoms of benzodiazepine overdose include anxiety, agitation, dizziness, confusion, nystagmus, slurred speech, altered mental state, amnesia, hypotension, and impaired cognition. Benzodiazepines can cause respiratory depression, which can be significantly increased when they are combined with other CNS depressants such as alcohol or opioids. Flumazenil is a specific antidote used to treat benzodiazepine overdoses but it comes with risks that may outweigh its potential benefits, so it is ideally used only to treat acute overdose in benzodiazepine-naïve individuals. Designer benzodiazepines are being detected with increasing frequency in hospitalizations, impaired driving cases, and overdose deaths.

### Other Classes

Around the same time that synthetic cannabinoids began appearing in the U.S., synthetic cathinones—sympathomimetic amines that are derivatives of methamphetamine and cathinone—gained popularity. Commonly called “bath salts” or “plant food,” the first generation of these products contained compounds such as methylenedioxypyrovalerone (MDPV), mephedrone, and methylone. Although the products were all classified under the street name of

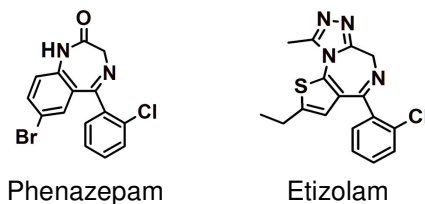


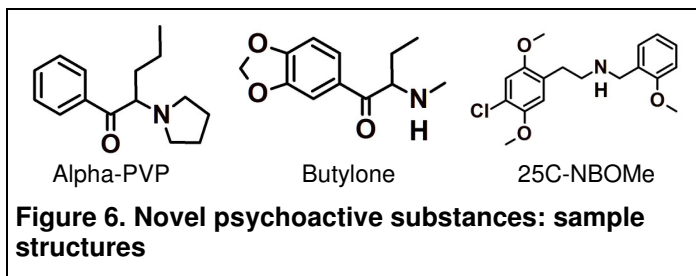
Figure 5. Novel benzodiazepines: sample structures

Table 3. Selected novel benzodiazepines: common name, chemical name, and regions where marketed

Phenazepam	Russia
7-bromo-5-(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one	
Etizolam	Japan India Italy
4-(2-chlorophenyl)-2-ethyl-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine	
Bromazepam	Europe Asia Africa
7-bromo-1,3-dihydro-5-(2-pyridinyl)-2H-1,4-benzodiazepin-2-one	
Flubromazepam	None
7-bromo-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one	
Flubromazepam	None
8-bromo-6-(2-fluorophenyl)-1-methyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine	
Delorazepam	Italy
7-chloro-5-(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one	
Diclazepam	None
7-chloro-5-(2-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one	
Clonazepam	None
6-(2-chlorophenyl)-1-methyl-8-nitro-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine	

“bath salts” and later “party pills” or “party powders,” the products contain a wide variety of stimulants, hallucinogens, enactogens, and dissociative anesthetics. Table 4 highlights some of the most popular compounds in these classes.

Synthetic cathinones are chemically similar to cathinone (a natural stimulant found in the khat plant) as well as the stimulants amphetamine and methamphetamine. Small changes to the structure of cathinone or amphetamine result in new drugs that behave very similarly pharmacologically, producing a spectrum of stimulant effects such as increased energy, tachycardia, and hypertension, while potentially evading detection in routine toxicology screens and



**Figure 6. Novel psychoactive substances: sample structures**

**Table 4. Selected novel psychoactive substances: common name, chemical name, and classification**

MDPV	Stimulant
1-(1,3-benzodioxol-5-yl)-2-(1-pyrrolidinyl)-1-pentanone	
Alpha-PVP	Stimulant
1-phenyl-2-(1-pyrrolidinyl)-1-pentanone	
Methylone	Enactogen
1-(benzo[d][1,3]dioxol-5-yl)-2-(methylamino)propan-1-one	
Ethylone	Enactogen
1-(benzo[d][1,3]dioxol-5-yl)-2-(ethylamino)propan-1-one	
Butylone	Enactogen
1-(1,3-benzodioxol-5-yl)-2-(methylamino)-1-butanone	
2C-B	Hallucinogen
4-Bromo-2,5-dimethoxyphenethylamine	
25C-NBOMe	Hallucinogen
2-(4-(chloro-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine	
5-MeO-DIPT	Hallucinogen
5-methoxy-N,N-bis(1-methylethyl)-1H-indole-3-ethanamine	
Methoxetamine	Dissociative
2-(ethylamino)-2-(3-methoxyphenyl)cyclohexanone	
3-MeO-PCP	Dissociative
1-[1-(3-methoxyphenyl)cyclohexyl]-piperidine	

avoiding classification as a scheduled drug.

As with synthetic cannabinoids, trends in the popularity of these compounds reflect the legislation controlling them. The Synthetic Drug Abuse Prevention Act of 2012 outlawed MDPV, so that drug was soon replaced by “flakka” (alpha-pyrrolidinopentiophenone or alpha-PVP). The use of alpha-PVP soared in 2014 and 2015, but after the U.S. pressured China to ban the sale of it and a number of other chemicals, it effectively disappeared. Methylone was a popular enactogen often used by attendees at electronic music festivals, but over time it has been replaced by ethylone, followed by butylone and dibutylone. Figure 7 traces the changes in the popularity of these drugs.

2C-B and 5-MeO-DIPT are just two of the many psychedelic substances described by Alexander Shulgin in his drug tomes. Of the novel psychoactive substances considered synthetic hallucinogens, most have appeared in only a handful of cases, with no substance gaining significant popularity (15,16).

### NBOMes

The possible exception to this lack of popularity is a group of substances called the NBOMes, which are the N-benzyl methoxy derivatives of the 2C series first described by Shulgin. The NBOMe compounds were originally developed as 5-HT<sub>2A</sub>-specific tags for PET-scanning brain research. The compounds were repurposed for recreational abuse due to their significant psychedelic and hallucinogenic effects. Because of their high affinity for the 5-HT<sub>2A</sub> receptor, the NBOMe drugs produce greater behavioral responses in animals and require lower doses to produce subjective effects in humans compared with their 2C-X counterparts (17).

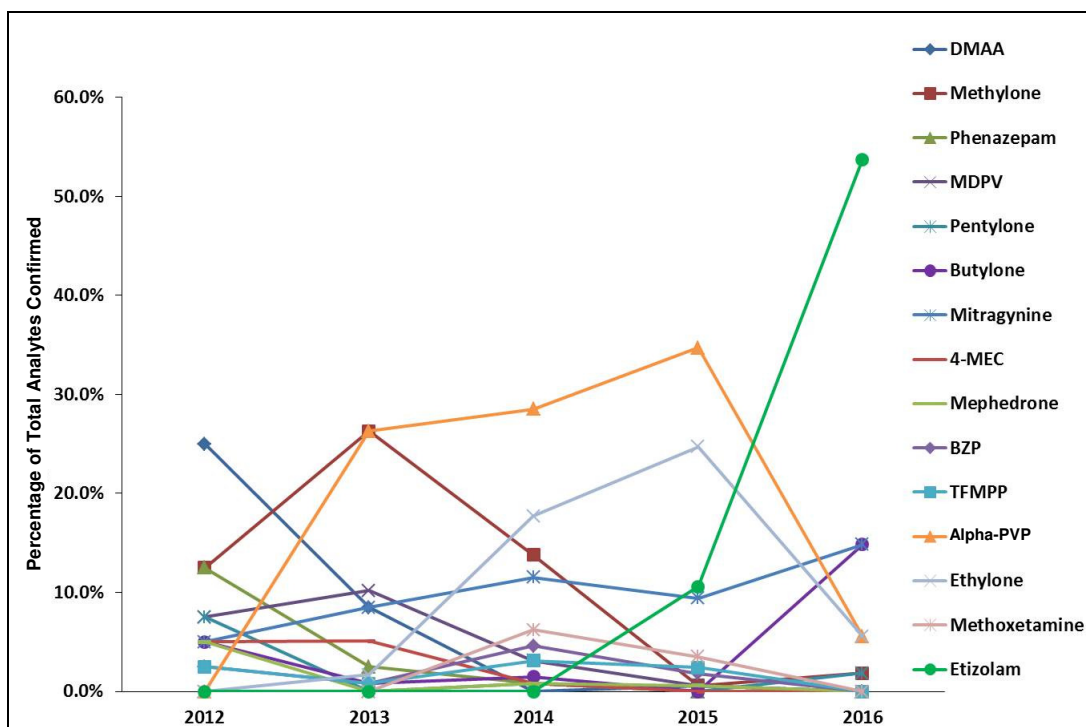
The subjective effects of the NBOMe compounds have never been studied in clinical trials, so all information regarding their effects comes from case reports and self-reports from users. The drugs are typically impregnated onto blotter papers, but are also sold as liquids for dropping or intravenous injection as well as powders to be insufflated or pressed into pills (18).

Due to the prevalence of blotter papers, the NBOMes have been commonly sold as either an alternative to LSD or as counterfeit LSD under the street name of acid. The blotter papers vary in the formulation and dosage of NBOMes, which poses a substantial risk to users, particularly naïve ones and those who are unaware they were sold NBOMe instead of LSD. Several individuals have died after using NBOMes, mostly teenagers and young adults (19–22).

Dissociative anesthetics such as methoxetamine and 3-methoxy-phencyclidine are structurally related to ketamine and phencyclidine and are reported to have similar effects. Although their mechanism of action is not completely understood, these compounds are antagonists at the NMDA receptor. Antagonism of this receptor results in hallucinations and dissociation.

### Challenges of NPS

Novel psychoactive substances present many challenges to clinical and forensic toxicologists, primarily because of the rapid evolution in the compounds available. From an analytical perspective, the wide range of chemical structures and classes means multiple tests must be run to determine which drugs



**Figure 7. Trends in positive rates for selected novel psychoactive substances from 2012 to 2106.**

Source: Data was extracted from the NMS Labs (Willow Grove, Pa.) laboratory information management system. Positive rate is the percentage of specimens tested that contained the analyte out of all samples that tested positive for at least one NPS compound in the scope.

are present. In addition, an up-to-date test menu is difficult to maintain because of the rapidly changing market and limited availability of analytical standards for new compounds. Laboratories must weigh the costs of new test development against the likelihood that the test will be needed for only a short time because of the frequent changes in patterns of use.

Although the novel benzodiazepines and fentanyl-related substances might be detected because of cross-reactions with routine urine drug screens, emergency department drug panels are not likely to detect other types of NPS. Even detected drugs can give difficult-to-interpret results because some novel benzodiazepines metabolize into other novel benzodiazepines and common prescription analytes. For example, diclazepam metabolizes to delorazepam, which in turn metabolizes to lorazepam. So a positive lorazepam test can result from the use of diclazepam, which tests do not detect. Therefore, clinical toxicologists may have to treat patients suffering from toxic effects of drugs without knowing the specific compound.

Another complicating factor is that most of these drugs have never been tested on animals, so there is limited information on them. This dearth of pharmacokinetic and pharmacodynamics data makes it diffi-

cult to assess the role of a designer drug in a clinical or postmortem case.

The changing legal landscape also poses challenges. There is a lack of standards to determine whether a drug meets the definition of an analog of a controlled substance, and therefore regulations are often patchwork. A comprehensive ruling to deal with the fast-paced evolution of designer drugs appears to be out of reach at this time.

New psychoactive substances are reaching the recreational drug market at a rate that outpaces the efforts of toxicology laboratories, drug monitoring agencies, law enforcement officials, and medical response personnel. Not only do new substances constantly replace those made illegal or that simply drop in popularity, but new classes of drugs

expand the population of people exploring new ways to get high or who are unwittingly supplied a designer substance as a substitute for their drug of choice. With the seemingly endless list of new substances, it appears that this designer drug movement has become a constant fixture in the drug landscape.

## Learning Objectives

After reading this article, the reader will be able to describe four categories of novel psychoactive substances and examples of drugs in each category. The reader will also be able to explain the challenges associated with the analysis of biological samples for novel psychoactive substances and the interpretation of the results.

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## Testing for Everolimus *Immunoassays Make Advances, But LC-MS/MS Is Still Preferred*

By Kamisha L. Johnson-Davis, PhD, DABCC, FACB

Everolimus is an immunosuppressant drug for prophylaxis of acute and chronic rejection in patients who receive renal or cardiac transplants. It is sold under the brand names Certican, Zortress, and Afinitor (1,2).

The Afinitor form has also been used in the field of oncology since 2009, when the U.S. Food and Drug Administration (FDA) approved its use for treating renal cell carcinoma after treatment with sunitinib or sorafenib has failed (3). The FDA has also approved its use for the treatment of subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex in patients who are not candidates for surgery and for use in combination therapy in some breast cancer cases.

### Mechanism of Action

Everolimus (Figure 1) is a 40-O-(2-hydroxyethyl) derivative of sirolimus (rapamycin), which is a macrocyclic lactone produced as an antifungal metabolite by the bacterial strain *Streptomyces hygroscopicus* (4). Everolimus suppresses the immune system by inhibiting T-cell activation and proliferation by forming a complex with an immunophilin to block the activity of a kinase, mammalian target of rapamycin (mTOR) (5). A serine/threonine kinase,

mTOR regulates T-cell growth, proliferation, protein synthesis, and transcription.

The mTOR inhibitors sirolimus and everolimus are often used in conjunction with a calcineurin inhibitor, such as cyclosporine A, because the drugs act synergistically for greater immunosuppression. However, the combination increases the risk of nephrotoxicity and opportunistic infections, so careful monitoring is needed to optimize therapy and minimize the risk of adverse effects. Table 1 lists the pharmacokinetic parameters of everolimus.

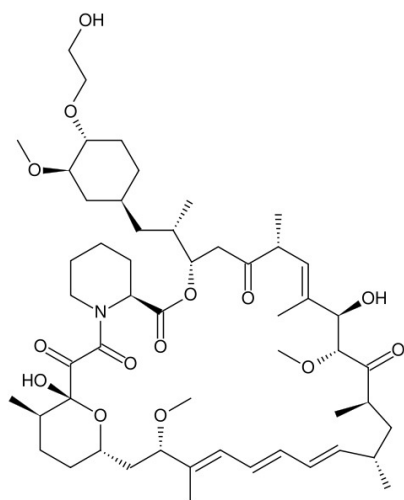
### Therapeutic Drug Monitoring

Drugs that have a narrow therapeutic range, inter-individual variability in pharmacokinetics, and consistent dose-response relationships are good candidates for therapeutic drug monitoring (TDM). Everolimus fits these characteristics. When used to prevent organ rejection or to treat SEGA, it has a narrow therapeutic index of 3–8 ng/mL (or 5–10 ng/mL without concomitant use of cyclosporine A) (6). Everolimus also poses the risk of adverse effects, including opportunistic infections, mouth ulcers, lymphoma and other malignancies, hyperlipidemia, proteinuria, and nephrotoxicity (1–3).

Everolimus is a substrate of CYP3A4/3A5, CYP2C8, and P-glycoprotein, so it can cause drug-drug interactions when administered with CYP3A4 inhibitors (cyclosporine A, ketoconazole, erythromycin, and verapamil) or inducers (rifampin, carbamazepine, and phenytoin) (1–3). These interactions can affect everolimus concentrations and therapeutic efficacy. Consequently, the dosage of everolimus needs to be adjusted to attain pre-dose (trough) concentrations within the therapeutic range. The frequency of everolimus TDM varies according to the patient's clinical condition—such as whether the patient is being treated after an organ transplant or for a condition such as renal cell carcinoma—as well as factors such as the need to monitor patient compliance (6).

### Assays for Everolimus TDM

Routine TDM is performed on trough whole blood collections. Liquid chromatography–tandem



**Figure 1. Everolimus**

Source: <http://prospect.rsc.org/blogs/cw/wp-content/uploads/2009/04/487px-everolimussvg.png>

**Table 1. Everolimus Pharmacokinetics**

Absorption: peak concentrations 1–2 hours post-dose
Volume of distribution: 4–9 L/kg
Plasma protein binding: ~74% in healthy subjects
Metabolism: substrate for CYP3A4, 3A5, 2C8, and P-glycoprotein with 11 inactive metabolites
Excretion: ~98% in bile and ~2% in urine
Half-life ( $T_{1/2}$ ): 26–38 hours
Steady-state concentration achieved in 4–7 days

mass spectrometry (LC-MS/MS) methods for everolimus quantification have been used since the late 1990s and are the currently recommended method.

Prior to the development of an everolimus immunoassay, some laboratories used sirolimus assays with a correction factor, which was an off-label use. However, in 2003, a fluorescent polarization immunoassay for everolimus (Innofluor Certican Assay System; Seradyn Inc., Indianapolis, Ind.) was introduced for use on the Abbott TDxFLx instrument (Abbott Diagnostics, Abbott Park, Ill.) (7). The assay had a limit of quantification (LOQ) of 2.0 ng/mL and a limit of detection (LOD) of 0.8 ng/mL.

A comparison between this immunoassay and LC-MS/MS demonstrated that the fluorescent polarization assay had a 20–30% positive bias because of cross-reactivity with inactive metabolites, such as hydroxyeverolimus, desmethyleverolimus, and dihydroxyeverolimus. A positive bias in results could be a problem for dose recommendations for optimal immunosuppression.

### QMS Immunoassay

In 2011, ThermoFisher Scientific launched an everolimus quantitative microsphere system (QMS) immunoassay, which is a homogeneous particle-enhanced turbidimetric technology (8). The assay is based on antibody competition between the everolimus-coated particle in the reagent and the drug in the patient's sample. If everolimus is not present, then the everolimus-coated particle in the reagent is agglutinated with anti-everolimus antibody. Agglutination decreases with the increase in everolimus concentration. The change in absorbance is monitored at 700 nm.

When this assay was evaluated on the Hitachi 917 analyzer in 2011, it had an LOQ of 1.3 ng/mL, an analytical measurement range of 1.5–20.0 ng/mL, and imprecision of  $\leq 13.3\%$ . Compared with LC-MS/MS, it had an average 11% positive bias, with a range from  $<10\%$  to 51.7% (8).

In 2014, the QMS Everolimus Assay was evaluated on the Ortho Vitros 5,1 Fusion analyzer (9). It had an LOD of 0.70 ng/mL, an analytical measurement range of 0.75–20 ng/mL, and imprecision of  $\leq 17.2\%$ . It still demonstrated a 27% positive bias in comparison with LC-MS/MS.

In 2015, an evaluation on an Abbott Architect ci4100 suggested that the QMS immunoassay results differed by -2.2 ng/mL to +5.2 ng/mL from LC-MS/MS results (10).

### Improving Agreement with LC-MS/MS

To minimize the positive bias from immunoassay results caused by cross-reactivity with inactive

metabolites, some vendors have created a calibration strategy based on assigning a value to calibrators and quality control samples. A value of about 70% of the gravimetric concentration seems to alleviate the approximately 30% positive bias in results in comparison to LC-MS/MS. However, when these assays are challenged by proficiency testing programs, which tend to spike samples with everolimus but not add metabolites, the results could trend 30% lower than LC-MS/MS results (6).

### Summary

In spite of the recent advances in immunoassay methods for everolimus analysis, consensus guidelines still recommend LC-MS/MS as the preferred method for determining everolimus concentrations, and that results should not be used interchangeably between immunoassays and LC-MS/MS (6).

### Learning Objectives

After reading this article, the reader will be able to describe the clinical indications for everolimus and the need for therapeutic drug monitoring. The reader will also be familiar with advances in testing for everolimus, particularly the performance of immunoassays.

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The author has nothing to disclose.

## Many AACC Meeting Sessions Cover Toxicology and TDM Topics

*By Christine Snozek, PhD, DABCC, FACB*

It's that time of year again—the AACC 2016 annual meeting will be held in Philadelphia from July 31 to August 4. There is a great lineup of speakers related to therapeutic drug monitoring and toxicology, including Thursday's plenary speaker, Dr. Marilyn Huestis, chief of the chemistry and drug metabolism section in the intramural research program at the National Institute on Drug Abuse. Dr. Huestis will be addressing laboratory and societal concerns related to medical marijuana.

And remember to mark your calendars for the TDM and Toxicology Division lunch on Monday from 12–2 pm.

Here are some highlights of sessions related to TDM, toxicology, and pharmacogenetics:

### Monday, August 1

42110/52210 *Ethanol Metabolites Testing in Non-Traditional Matrix Types: Case Studies*. Brown bag, 7:30–8:30 am and repeated at 12:30–1:30 pm.

72414 *Be Prepared! Sample Preparation Strategies for Multiple Matrices in the LC-MS/MS Clinical Toxicology Laboratory*. Mid-day short course, 12:30–2 pm.

72415 *NACB LMPG on Laboratory Testing to Support Pain Management*. Mid-day short course, 12:30–2 pm.

72222 *When Toxicology Results and Clinical Presentation Do Not Correlate: How to Communicate With Clinicians and Guide Further Testing Decisions*. Afternoon short course, 2:30–4 pm.

### Tuesday, August 2

43104/53204 *Therapeutic Drug Management in Pregnant Patients*. Brown bag, 7:30–8:30 am and repeated at 12:30–1:30 pm.

43107/53207 *How People Try to Beat Drug Testing and Defend Positive Results*. Brown bag, 7:30–8:30 am and repeated at 12:30–1:30 pm.

43118/53218 *Pharmacogenetics in the Clinical Laboratory: Opportunities and Challenges*. Brown bag, 7:30–8:30 am and repeated at 12:30–1:30 pm.

33102 *Recently Revised CLSI Protocol C52; "Toxicology and Drug Testing in the Clinical Laboratory—Approved Guideline."* Morning symposium, 10:30 am–noon.

### Wednesday, August 3

44103/54203 *Mass Spectrometry in the Clinical Lab: Applications for Emergency Toxicology*. Brown bag, 7:30–8:30 am and repeated at 12:30–1:30 pm.

44107/54207 *Monoclonal Antibodies as Therapeutic Agents*. Brown bag, 7:30–8:30 am and repeated at 12:30–1:30 pm.

44114/54214 *Hair as a Long Term Adherence Marker for HIV Medications*. Brown bag, 7:30–8:30 am and repeated at 12:30–1:30 pm.

44122/54222 *Evaluation of Time-of-Flight High Resolution Mass Spectrometry for Broad Spectrum Drug Screening in Urine*. Brown bag, 7:30–8:30 am and repeated at 12:30–1:30 pm.

44128/54228 *Common Pitfalls and Misinterpretations of Urine Drug Testing*. Brown bag, 7:30–8:30 am and repeated at 12:30–1:30 pm.

34101 *Oral Abstract Session: What's New in Toxicology and Therapeutic Drug Monitoring*. Morning symposium, 10:30 am–noon.

74214 *Sampling, The Next Generation: Emerging Clinical Mass Spec Applications in Alternative Matrices*. Afternoon short course, 2:30–4 pm.

74217 *Oral Fluid in the Clinical Toxicology Laboratory: Ready for Prime Time?* Afternoon short course, 2:30–4 pm.

### Thursday, August 4

15001 *The Great Cannabis Experiment: Medical Miracle? Harmless High? Civil Consequences?* Plenary session, 8:45–10:15 am.

35102 *Marijuana from the Street to the Clinic: Medicalization, Legalization, and Unintended Consequences*. Morning symposium, 10:30 am–noon.

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### Learning Objectives

Learning objectives vary by article, but in general, after completing *Clinical & Forensic Toxicology News*, the reader will be able to:

- Describe emerging and changing trends in drug abuse, including new designer drugs, usage patterns, and contaminants/adulterants.
- Identify potential analytes (drugs, metabolites, biomarkers) of clinical and/or forensic significance.
- Evaluate methodologies for their utility and limitations relative to the needs of toxicology labs.
- Discuss relevant regulations, such as analytical performance requirements, or the legality of new drugs of abuse.
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- Describe the medical implications of drug abuse, toxicity associated with therapeutic agents, and exposure to other toxicants.

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