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Hans H. Maurer
Simon D. Brandt *Editors*

New Psychoactive Substances

Pharmacology, Clinical, Forensic and Analytical Toxicology

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Preface

Historically, the legislative control of substances with perceived desired psychoactive effects has always triggered a search for non-controlled alternatives, and the appearance of psychoactive substances of predominantly synthetic origin can be traced back to these efforts. In the last decade, so-called new psychoactive substances (NPS) exploded into the consciousness of policy makers, researchers, practitioners, as well as the general public. NPS are typically viewed as substances not listed in the United Nations Single Convention on Narcotic Drugs, 1961, as amended by the 1972 Protocol, or the United Nations Convention on Psychotropic Substances, 1971, that may pose public health or social risks similar to the substances listed therein. Driven by globalization, easy access to NPS, the striking number of substances, their chemical diversity, and the realization that ideas for large-scale production originated from the – sometimes forgotten or otherwise unexplored – scientific literature, along with the growing numbers of life-threatening poisonings and other harms, have moved the NPS phenomenon firmly onto the policy agenda. Consequently, a variety of legislative and other policy responses have been formulated throughout the globe in an effort to protect public health.

As the dust is beginning to settle, it is now clear that the use of NPS has graduated somewhat from psychonautic explorations of substances obtainable from Internet retailers to a more complex phenomenon. For example, involvement of crime groups has led to NPS being increasingly sold, sometimes surreptitiously, on the "illicit" market, including as falsified (fake) medicines – which can have disastrous consequences. Psychoactive drugs have to be seen as commodities, which means that an overlap exists between "traditional" substance users and markets normally attracting the attention of user groups interested in health and image and performance enhancement. In this respect, globalized trade, electronic forms of communication, effective and cheap shipping, and contract manufacturing organizations available across the globe have placed the NPS phenomenon neatly within a larger phenomenon that encapsulates the areas associated with "designer" medicines, pharmaceuticals, and dietary supplements whereby novel analogs, also frequently originating from older scientific literature, are available for purchase by the sometimes unsuspecting public. New ways of masking detection and identification of NPS and the development of new dosage forms are also being devised. In the latter case, the sale of nasal sprays and e-liquids containing fentanyl derivatives raises

concerns about the spread of such substances to new user groups. Recent years have also witnessed increasing numbers of outbreaks of severe poisonings associated, for example, with synthetic cannabinoid receptor agonists (SCRAs) and synthetic opioids.

However, reflecting the highly dynamic nature of the market, detailed information about the epidemiology of NPS use, including prevalence, is still limited. It is also unclear whether the rate at which new appearances appear on the market will be sustained. What does emerge is that there is a greater need for protecting both people who use these substances and the broader public health.

The content of this book has been assembled to serve scientists, scholars, healthcare providers, law enforcement, policy makers, and people who use drugs and who are fascinated by and exposed to the multilayered facets of the NPS phenomenon. Its highly dynamic nature means that this can only be a snapshot, but it is hoped that readers will get a taste of diverse perspectives provided by the contributing authors and how this information helps to complement the knowledge available on "traditional" psychoactive substances that still dominate the market.

In Part I, Evans-Brown and Sedefov set the stage by describing the origins of NPS and giving an overview of the situation in Europe from the perspective of the early warning and risk assessment activities conducted by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) that allows the European Union to rapidly detect, assess, and respond to public health and social threats caused by these substances. Then Tettey et al. from the United Nations Office on Drugs and Crime (UNODC) introduce the reader to the important activities being coordinated at the global level. On an individual level, people interested in the effects of NPS typically turn to electronic forms of communication for information, such as online discussion boards. At the same time, Passie and Brandt review the rich tradition of self-experiments with psychoactive substances carried forward by scientists and therapists for over a century, which shows that systematic approaches have been available that explored the nature of drugs and drug experience. In most cases, data from self-experiments are the only source on the clinical effects, as controlled human studies in this context are normally not carried out.

Part II is dedicated to providing an overview of the pharmacology of representative examples of commonly encountered NPS. This section begins with synthetic cathinones (Baumann and coworkers) where it is shown that some of the structural features are associated with monoamine transporter-mediated release of neurotransmitters, whereas others direct their activity toward uptake inhibition. Simmler and Liechti follow on with the coverage of amphetamine- and 3,4-methylenedioxymethamphetamine (MDMA)-like NPS. SCRAs are possibly the most diverse and perplexing class of substances that have attracted significant attention, not least from a public health perspective because of the large number of outbreaks of mass poisoning they have caused. The challenge of digesting this complex topic has been taken on by Banister and Connor, who provide two chapters on the origins and evolution of these substances from the viewpoint of molecular pharmacology. At the same time, the emergence of new synthetic opioids on the streets has caused particular concerns regarding their association with significant

numbers of life-threatening poisonings and fatalities. The overview presented by Beardsley and Zhang examines three synthetic opioids (U-47,700, MT-45, and acetylfentanyl) as representative examples belonging to three chemical classes. An increasing number of benzodiazepine-based NPS that predominantly originated from early scientific explorations carried out decades ago have resurfaced in recent years. While sold as substances in their own right, they have also been seen as fake diazepam and alprazolam. Moosmann and Auwärter provide insights into the number and types of substances that have been encountered. Classic serotonergic psychedelics, including psilocybin and lysergic acid diethylamide, are progressively explored in a range of clinical investigations. Clinton Canal offers a detailed discussion on preclinical experimental approaches for studying mechanisms of action of these substances, classic and new. The second part of this book finishes with two contributions provided by Wallach and Brandt who tackle the topic related to dissociative drugs represented by phencyclidine (PCP), 1,2-diarylethylamine-, and ketamine-based NPS.

In Part III, the attention is turned toward the clinical, forensic, and analytical toxicology of NPS. This area of work is especially important since the experiences gained in this field can not only increase the understanding of NPS effects on humans but also play a central role in the detection of harms for early warning systems. This section of the book commences with an overview of recent developments in the field of bioanalysis presented by Wagmann and Maurer who include topics related to sample preparation, methods of analysis and detection, data evaluation, and pitfalls. This is then supplemented by a contribution of Markus Meyer who offers an update on the toxicokinetics of NPS that considers the period between May 2016 and November 2017. From a European perspective, an important contribution to early warning was made by the STRIDA project that monitored the occurrence of poisonings linked to NPS in Sweden. Here, Helander and Bäckberg offer an overview of their experience with analytically confirmed poisonings presenting in hospital emergency departments and intensive care units that occurred during a ~6-year period from 2010 to early 2016 and which also included about 2,600 cases of suspected NPS intoxication. A common challenge experienced by healthcare professionals when dealing with adverse effects associated with NPS use is that information about drug identity is typically not available when it comes to a clinically meaningful time frame. Hill and Dargan inform the reader that clinicians aim for identifying the clinical toxidrome based on the clinical features observed at presentation. The authors provide an overview of the different sources that may inform the understanding of patterns of acute toxicity with NPS and review the existing literature. The most tragic outcome associated with NPS toxicity is death. Kronstrand et al. review the circumstances, antemortem symptoms, and toxicological findings that have led to death following use of NPS, thus offering a forensic toxicology perspective. The authors conclude that deaths attributed to NPS significantly increased during the last 2 years and that this might have been a reflection of a shift from SCRAs and cathinones to the more toxic and dangerously potent fentanyl derivatives, which adds to the general debate about the perceived shift from illicit opioids/diverted opioids to some of these new analogs. In the final contribution,

Ort et al. demonstrate how the chemical analysis of wastewater adds an important piece of the epidemiological puzzle in the effort to understand community-wide drug use.

The editors are grateful to the HEP editors Veit Flockerzi and Jim Barret for providing us with the opportunity to compile this book and the team at Springer especially Susanne Dathe and Anand Venkatachalam for their support and constructive collaboration. Finally, the editors would like to express their gratitude to all the authors who contributed to this book, which would have not been possible without their willingness to spend their valuable time on writing the chapters.

Homburg (Saar), Germany — Hans H. Maurer
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Contents

Responding to New Psychoactive Substances in the European Union: Early Warning, Risk Assessment, and Control Measures

Michael Evans-Brown and Roumen Sedefov

Contents

Abstract

New psychoactive substances (NPS) are drugs that are not controlled by the United Nations international drug control conventions of 1961 and 1971 but that may pose similar threats to public health. Many of them are traded as "legal" replacements to controlled drugs such as cannabis, heroin, benzodiazepines, cocaine, amphetamines, and 3,4-methylenedioxymethamphetamine (MDMA). Driven by globalization, there has been a large increase in the availability and, subsequently, harms caused by these substances over the last decade in Europe. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is monitoring more than 670 NPS that have appeared on Europe's drug market in the last 20 years, of which almost 90% have appeared in the last decade. While some

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recent policy responses have been successful in reducing availability and sales of these substances in some settings – such as "legal highs" and "research chemicals" sold openly in the high street and online – and there are signs that growth in the market is slowing, new challenges have emerged. This includes monitoring a growing number of highly potent substances – including 179 synthetic cannabinoid receptor agonists and 28 fentanils – that can pose a high risk of life-threatening poisoning to users and can cause explosive outbreaks. This chapter briefly traces the origins of NPS, provides an overview of the situation in Europe, and discusses the work of the EMCDDA as part of a legal framework of early warning, risk assessment, and control measures that allows the European Union to rapidly detect, assess, and respond to public health and social threats caused by these substances.

Keywords

Adulteration · Benzodiazepines · Designer drugs · Dietary supplements · Early warning systems · Fentanils · Globalization · Legal highs · Misbranding · New psychoactive substances · Opioids · Outbreaks · Preparedness · Public health policy · Risk assessment · Synthetic cannabinoid receptor agonists · Synthetic cathinones

Acronyms and Names of the Discussed New Psychoactive Substances (NPS) and Controlled Drugs

α-Methylfentanyl	*N*-[1-(1-Methyl-2-phenylethyl)-4-piperidinyl]-*N*-phenyl-propanamide
Δ^9-THC	(6a*R*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-dibenzo[*b*,*d*]pyran-1-ol (Δ^9-tetrahydrocannabinol)
AB-CHMINACA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide
Acetylfentanyl	*N*-Phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]-acetamide
Acryloylfentanyl	*N*-Phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]prop-2-enamide (acrylfentanyl)
ADB-CHMINACA	*N*-[(2*S*)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide
Carfentanil	Methyl 1-(2-phenylethyl)-4-[phenyl(propanoyl)amino] piperidine-4-carboxylate
CUMYL-4CN-BINACA	1-(4-Cyanobutyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
Cyclopropylfentanyl	*N*-Phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl] cyclopropanecarboxamide

4F-iBF	*N*-(4-Fluorophenyl)-2-methyl-*N*-[1-(2-phenylethyl) piperidin-4-yl]propanamide (4′-fluoroisobutyrylfentanyl)
4-Fluorofentanyl	*N*-[4-Fluoro-1-(2-phenylethyl)piperidin-4-yl]-*N*-phenylpropanamide
5F-MDMB-PINACA	Methyl (2*S*)-2-{[1-(5-fluoropentyl)-1*H*-indazole-3-carbonyl]amino}-3,3-dimethylbutanoate (5F-ADB)
Fentanyl	*N*-Phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]-propanamide
Furanylfentanyl	*N*-Phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]furan-2-carboxamide (2-furanylfentanyl)
HU-210	3-(1,1′-Dimethylheptyl)-6aR,7,10,10aR-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[b,d]pyran-9-methanol
JWH-018	(1-Pentyl-1*H*-indol-3-yl)-1-naphthalenyl-methanone
LSD	(8β)-*N*,*N*-diethyl-6-methyl-9,10-didehydroergoline-8-carboxamide (*d*-lysergic acid diethylamide)
MDMA	1-(2*H*-1,3-Benzodioxol-5-yl)-*N*-methylpropan-2-amine (3,4-methylenedioxymethamphetamine)
3-Methylfentanyl	*N*-[3-Methyl-1-(2-phenylethyl)piperidin-4-yl]-*N*-phenylpropanamide
Methoxyacetylfentanyl	2-Methoxy-*N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]acetamide
MDMB-CHMICA	Methyl (2*S*)-2-{[1-(cyclohexylmethyl)-1*H*-indole-3-carbonyl]amino}-3,3-dimethylbutanoate
THF-F	*N*-Phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]oxolane-2-carboxamide (tetrahydrofuranylfentanyl)

1 Introduction

This world is increasingly complex and interconnected. New risks are constantly emerging that can threaten public health; some are familiar, others are novel. Driven by globalization, the serious cross-border threats to health from the (re)emergence and spread of infectious diseases (such as Zika, yellow fever, and Ebola) and the growing market of unlicensed and falsified (fake) medicines are just two examples of policy areas that have required extensive changes to their regulatory systems, both at the level of legislation and implementation, in order to manage these risks more effectively (Directive 2011/62/EU 2011; WHO 2018; Decision No 1082/2013/EU 2013). Drug markets have not been immune to these global changes either, with new psychoactive substances (NPS) providing an important case study of how new threats can rapidly emerge and establish themselves in society (EMCDDA 2016a).

NPS are a broad range of drugs that are not controlled by the United Nations international drug control conventions of 1961 and 1971 but that may pose similar threats to public health (Single Convention on Narcotic Drugs 1961; Convention on Psychotropic Substances 1971; Council Decision 2005/387/JHA 2005; Regulation (EC) No 1920/2006). Many of them are traded as "legal" replacements to established controlled drugs such as cannabis, heroin, benzodiazepines, cocaine, amphetamines, and MDMA (EMCDDA 2016a, 2018a).

Over the last decade, there has been a large increase in these substances as globalization and new technologies, such as the Internet, have allowed them to be produced, sold, and supplied on an industrial scale (Griffiths et al. 2013; Evans-Brown and Sedefov 2017). This has led to a range of challenges for public health policy and practice. At least initially, national drug control laws struggled to keep up with a steady flow of new substances appearing – their open sale in shops on the high street and Internet often adding to this problem (EMCDDA and Eurojust 2016; EMCDDA 2018a). The consumer base has also grown in parallel with the range of substances and products that were offered. It includes people who use them recreationally; those with problematic drug use, who self-medicate; as well as people wanting to look better, get fitter, or enhance their performance at school or work (Griffiths et al. 2013). Reports of severe and fatal poisonings involving these substances have also grown substantially (EMCDDA 2018b).

Nonetheless, the picture across Europe (which has more than 500 million inhabitants) is complex as the situation differs widely both geographically and over time. In addition, the capability and capacity to detect and report events that are important for early warning activities (such as poisonings that are confirmed by laboratory testing) can also differ, meaning that there is both under-detection and under-reporting in some areas and settings. More generally, understanding the epidemiology of NPS remains weak. This includes problems with estimating the prevalence of use of new substances, which can be a complex and frustrating task because of the large number of substances and products that are available but also because of the highly dynamic nature of the market. In many cases, individuals do not actually know what new substance they are using, while in other cases they may not even realize that they are using a new substance; for a discussion of these issues as well as review of prevalence data, the reader is referred to Sumnall (2016).

While some of the recent policy responses have been successful in reducing the availability of NPS in some settings – such as measures aimed at reducing the open sale of "legal high"-type products in high-street shops – the overall continued availability of new substances is driving greater complexity into the drug situation. This includes major new challenges, such as an increase in the number of highly potent substances appearing on the market. These pose a high risk of life-threatening poisoning to users, can cause explosive outbreaks, and, in some circumstances, may pose a risk of occupational exposure to personnel (EMCDDA 2018b).

In Europe, a three-step legal framework of early warning, risk assessment, and control measures allows the European Union to rapidly detect, assess, and respond to public health and social threats caused by these substances. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is responsible for the first two

steps in this system, namely, operating the EU Early Warning System with Europol (the European Union Agency for law enforcement cooperation) and conducting risk assessments, whereas the European Commission, the Council of the European Union, and the European Parliament are responsible for control measures (Council Decision 2005/387/JHA 2005; Regulation (EC) No 1920/2006).

This chapter briefly traces the origins of new psychoactive substances, provides an overview of the situation in Europe, highlights some of the recent major concerns and challenges using the synthetic cannabinoid receptor agonists and the fentanils as case studies, and discusses how the European Union (EU) is responding to this threat. In doing so, information is drawn from material and approaches developed by the EMCDDA's early warning and risk assessment activities that aim to support and strengthen national- and EU-level preparedness and responses to these substances.

2 The Origins of New Psychoactive Substances (NPS)

Humans have used psychoactive substances (drugs) for thousands of years. Throughout this time, they have been used for medicinal and spiritual purposes, for relaxation, pleasure, and curiosity, as well as to enhance creativity and performance. Initially, most of these substances were from the use of plants, such as the opium poppy, ephedra, coca, peyote, and cannabis (Berridge and Edwards 1981; Schivelbusch 1993; Courtwright 2002; Sneader 2005; Miller 2014; Richards 2016).

As the field of organic chemistry developed during the nineteenth and twentieth centuries, scientists were able to isolate the active substances from such plants. It also allowed them to determine their chemical structures, manipulate them, and develop a range of new substances (Sneader 2005). Crude opium from the poppy was purified to give morphine, whose structure was tweaked to give diacetylmorphine – a more potent opioid that was sold from the 1890s onward under the trade name heroin and marketed, incorrectly as it turned out, as a "nonaddictive" replacement to morphine (Courtwright 2002). Ephedra led to the isolation of ephedrine, which was subsequently used to make amphetamine – a potent stimulant that was extensively overprescribed in the 1950s in America for weight loss and mood disorders (Rasmussen 2008a, b). Other developments in the field of chemistry led to the discovery of additional sources that could be used as the building blocks for new chemicals, leading, overall, to the invention of a large range of psychoactive substances (Sneader 2005).

The goal of much of this work was to develop new and better medicines. While a relatively small number were successfully commercialized as such, many others fed into the research cycle, being used as pharmacological and clinical tools to study the body, provide insights into disease states, and as chemical templates for developing new types of substances. The results of this ongoing work are cataloged in the scientific and patent literature that provides the blueprints and recipes for making thousands of psychoactive substances (Sneader 2005).

Of the substances that were used as medicines, many spread beyond the sphere of medicine – driven by consumer demand, weak regulation, and wider social and

cultural changes (Tone 2008; Herzberg 2010, 2012; Rasmussen 2012; Berridge 2013). As concerns grew during the twentieth century over the health and social harms caused by these medicines, control measures were increasingly introduced or tightened in an attempt to reduce their availability and limit their harms (Brunn 1975; Musto 1973; McAllister 1999). In many cases illicit markets sprang up, some of which were supplied by diverted medicines or from illicit laboratories. In addition, attempts were made to get round these controls. For example, after morphine became a controlled drug in the 1920s, pharmaceutical companies produced vast quantities of the non-controlled morphine esters benzylmorphine and acetylpropionylmorphine to sell on the illicit opioid market (Anonymous 1953); in the 1960s, following the discovery and synthesis of THC, which is the main psychoactive constituent of cannabis, raids on illicit laboratories found the ingredients and recipes to make "synthetic marijuana" (New York Times 1968), while from the late 1970s onward, the fentanils (highly potent derivatives of the opioid analgesic fentanyl) were made in illicit laboratories and sold as heroin or "synthetic heroin" to unsuspecting users (Baum 1985; Henderson 1988, 1991).

Until the 1960s, most of the substances that did appear on the illicit drug market were medicines. After that, a handful of the other substances also began to appear as word of their effects escaped research laboratories and spread to small groups of people who were keen to experiment with them. Some failed to catch on further and remained "chemical curiosities," usually because the pharmacological effects were of interest only to a small number of people or because of the unpleasant or harmful effects that they produced (Meyers et al. 1968; Shulgin 1975; Cooper 1988). Others, such as LSD and MDMA (or "ecstasy" as it is better known), spread widely, being produced in hobbyist and illicit laboratories and eventually became important substances for the drug market. Many of these substances have fascinating and sometimes long and complex stories that tell of how they came to be discovered and used within society (Beck and Rosenbaum 1994; Collin 1997; Reynolds 1999; Dyck 2008; Morris and Wallach 2014; Passie and Benzenhofer 2016, 2018).

So, the appearance and use of "new" substances is not a new chapter in the history of drug use (Sumnall et al. 2011; Brandt et al. 2014). While diverted medicines, such as pregabalin (Baird et al. 2014; Häkkinen et al. 2014; McNamara et al. 2015), and substances produced in illicit laboratories, such as 4-methylamphetamine (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) 2014a), continue to be important sources of new substances, what is new is the dramatic increase in the speed and quantity in which new substances have appeared on the drug market over the last decade or so. The handful of substances has turned into hundreds, as entrepreneurs and crime groups have systematically exploited the literature (Fig. 1) and mass produced a large range of new substances and branded products, leading to huge growth in the market (Griffiths et al. 2013; EMCDDA 2016a, 2018a, b).

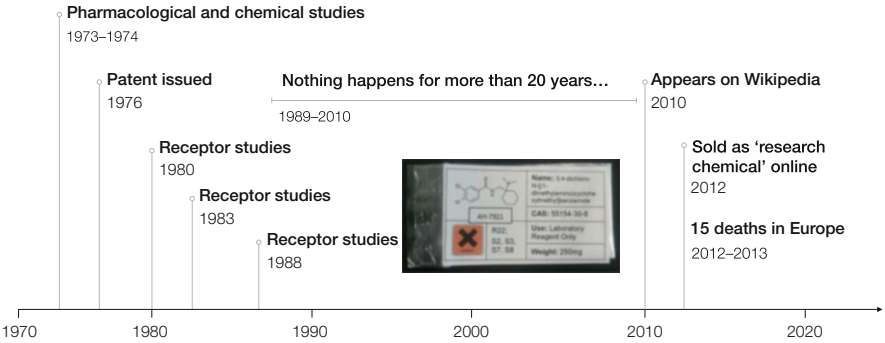


Fig. 1 AH-7921 is just one of the hundreds of substances from the scientific and patent literature that entrepreneurs have exploited in the last decade. The substance is a structurally unique synthetic opioid analgesic that was invented during the mid-1970s as part of the search for a "better morphine" by the pharmaceutical company Allen and Hanburys Limited (that eventually became part of GlaxoSmithKline). Known by its company code name, AH-7921 was researched in nonclinical studies but was not commercialized as a medicine. Twenty years after the last research paper on it was published, a Wikipedia page for the substance was created that highlighted its opioid pharmacology including its similarities to morphine. Analysis of a sample purchased from an online vendor made in July 2012 confirmed that AH-7921 was being sold openly in Europe under the guise of being a "research chemical." Users were also discussing it online as a "legal opioid." By the end of 2013, the substance had been detected on the drug market in 9 countries in Europe and involved in at least 15 deaths. Vendors based in Europe and China were offering up to multi-kilogram quantities of the substance. Similar to other opioids, AH-7921 can pose a risk of life-threatening poisoning from respiratory depression. Following a risk assessment by the EMCDDA in 2014, AH-7921 was subject to control measures in Europe (EMCDDA 2014b). In 2015, it was also controlled by the international drug control system. A total of 38 new synthetic opioids have been detected in Europe since 2009. © European Monitoring Centre for Drugs and Drug Addiction, 2018. Reproduction of the timeline is authorized provided the source is acknowledged. Image of AH-7921 kindly provided by Dr. Roland Archer, States Analyst, States of Guernsey. © Dr. Roland Archer

3 The Situation in Europe

By the end of 2017, the EMCDDA was monitoring more than 670 new psychoactive substances that have appeared on Europe's drug market over the past 20 years. Almost 600 (90%) of these have appeared in the last decade, including 51 substances that were reported for the first time during 2017 (Fig. 2). They include a broad range of substances, including synthetic cannabinoid receptor agonists (SCRAs), synthetic cathinones, opioids, benzodiazepines, phenethylamines, and tryptamines (Fig. 2). While the situation differs widely across Europe, this dramatic growth is also reflected in large increases in seizures made by law enforcement over this period as well as substantial increases in reports involving severe and fatal poisonings (EMCDDA 2018a, b).

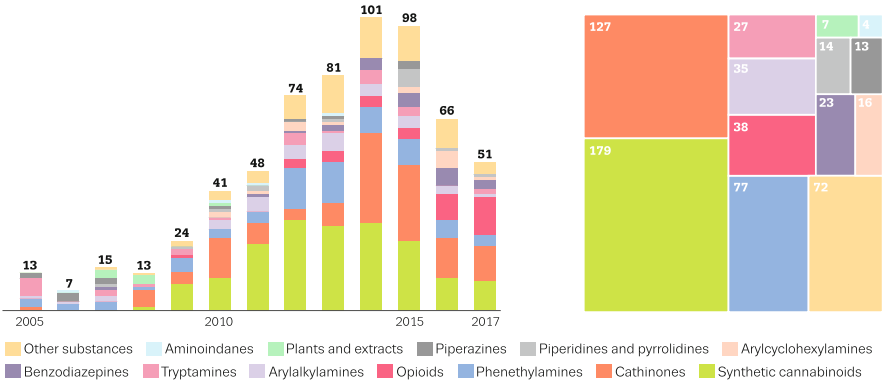


Fig. 2 New psychoactive substances notified to the EU Early Warning System for the first time 2005–2017: number per year (left) and total number per category (right). European Monitoring Centre for Drugs and Drug Addiction, 2018. Reproduction is authorized provided the source is acknowledged

During 2016, more than 70,000 seizures of new substances that weighed 4.1 tons were reported to the EU Early Warning System by law enforcement agencies from across Europe (Fig. 3). Similar to recent years, the seizures were dominated by SCRAs and synthetic cathinones, which, together, accounted for around 80% of the total number and quantity of new substances reported during the year (Figs. 4 and 5). The larger number of seizures reported for SCRAs reflects their use as "legal" replacements to cannabis, which is the mostly commonly used drug in Europe. The larger number of seizures reported for the synthetic cathinones reflects their use as "legal" replacements for large markets in cocaine, amphetamines, MDMA, and other controlled stimulants (EMCDDA 2018a, b).

Seizures of new psychoactive substances reported to the EU Early Warning System must be understood as minimum values, as data are drawn from case reports rather than monitoring systems. Reports are influenced by a range of factors such as increasing awareness of new substances, their changing legal status, law enforcement capacities and priorities, and the reporting practices of law enforcement agencies. The data are not directly comparable to the data on established controlled drugs. The data also include a small number of new psychoactive substances that have been recently controlled internationally under the UN drug control conventions.

3.1 Production, Marketing, and Supply

The growth in the market observed in recent years has only been possible because of a shift in production from small-scale illicit laboratories in Europe to chemical and pharmaceutical companies operating predominantly in China that are capable of making these substances on an industrial scale. This has been driven by globalization and new technologies, with increasing expertise and capacity in the Chinese science

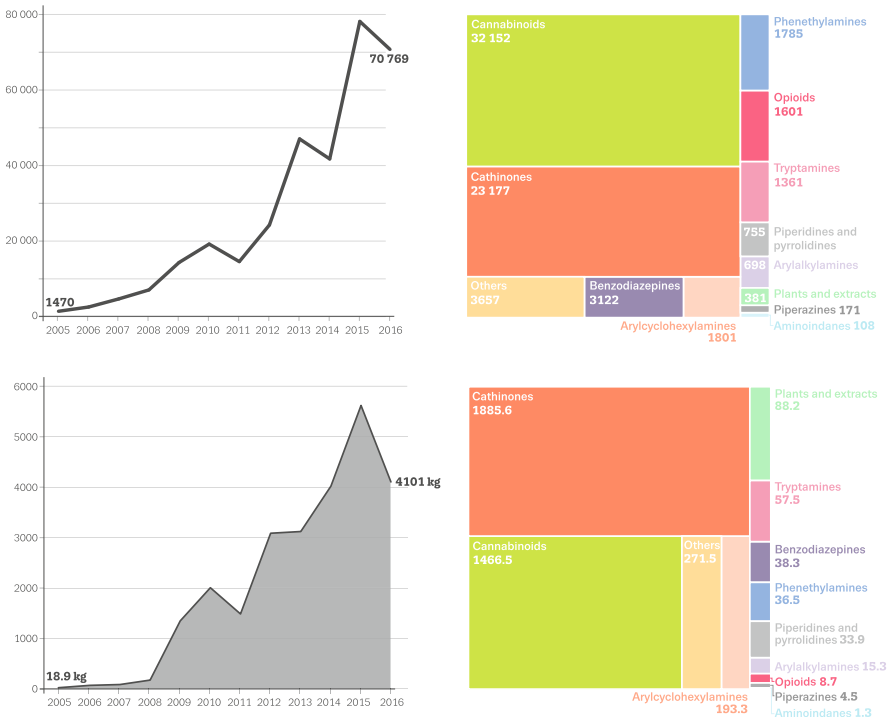


Fig. 3 Number and quantity of new psychoactive substance seized by law enforcement reported to the EU Early Warning System: trends and distribution by category in 2016. © European Monitoring Centre for Drugs and Drug Addiction, 2018. Reproduction is authorized provided the source is acknowledged

and technology economy, low labor costs, the Internet, and cheap and efficient shipping (Smil 2010; Stearns 2011; Morris 2012; Levinson 2016). Using online marketplaces to advertise their catalog, the companies offer a diverse range of highly pure products in quantities that range from a few milligrams to tens or even hundreds of kilograms (Halford 2015; Deprez et al. 2018). Furthermore, some offer a custom chemical synthesis service. Other ingredients, equipment (such as tableting and packaging machines), and packaging materials that are needed to make products (see below) may also be sourced from companies based in China. Companies based in India can also be an important source of NPS, particularly those substances that are also classed as medicines (such as modafinil and tramadol).

From China, the substances are shipped to wholesalers, retailers, and dealers in Europe by express mail and courier services (Fig. 6), whereas larger quantities ship by air and sea cargo (Fig. 7) (EMCDDA 2016a). Consignments are often misdeclared as common goods of low value, including foodstuffs and other chemical products, in order to conceal their true nature and avoid suspicion by customs and border forces (EMCDDA 2016a). This includes the case of the opioid acetylfentanyl – an analog of fentanyl that was linked to 29 deaths in Europe during 2015 – where consignments

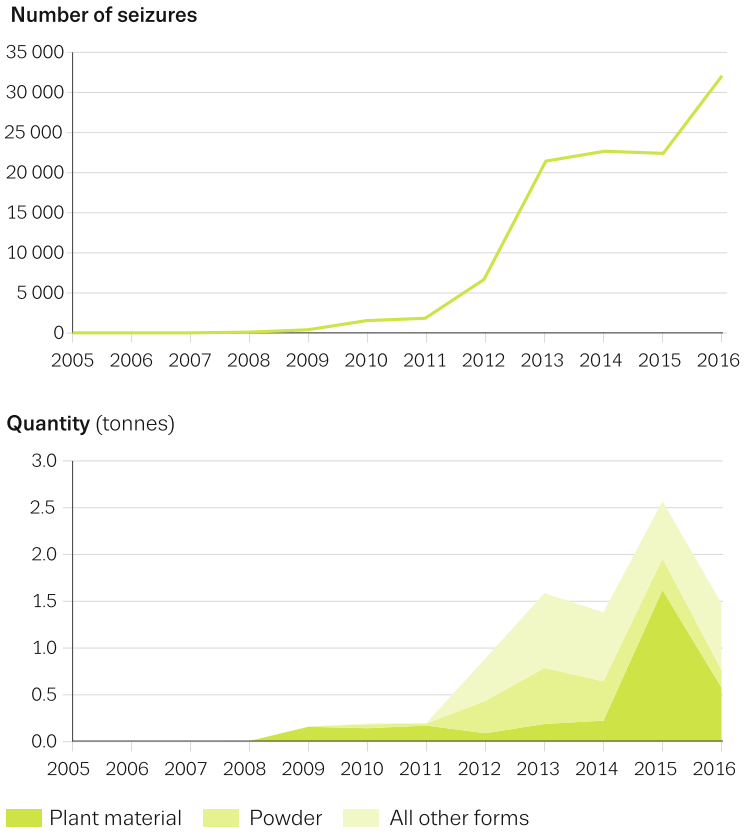


Fig. 4 Seizures by law enforcement of SCRAs reported to the EU Early Warning System: trends in number of seizures and quantity seized, 2005–2016. © European Monitoring Centre for Drugs and Drug Addiction, 2018. Reproduction is authorized provided the source is acknowledged

were misdeclared as a commonly used adhesive called "hot melt powder" (EMCDDA 2016b). Suppliers and importers may also deliberately route NPS to specific air and seaports in Europe where the substances are not controlled in order to reduce the chance of seizure (EMCDDA 2016a).

In Europe, some NPS are then processed into branded products that are sold openly or under the counter in shops as well as online (Griffiths et al. 2013; EMCDDA 2016b; Södertörns Tingsrätt 2018). At least initially, it was these products that characterized the growth in the market, with the three main categories being marketed as "legal highs," "research chemicals," and "dietary supplements." The products were designed to be attractive to consumers, avoid the attention of regulators, and sidestep consumer protection laws. "Legal highs" were packaged in colorful packaging often suggestive of controlled drugs or psychoactive effects and were usually labeled as "not for human consumption" and as advertised as "incense," "plant food," or "novelty items," while "research chemicals" were labeled

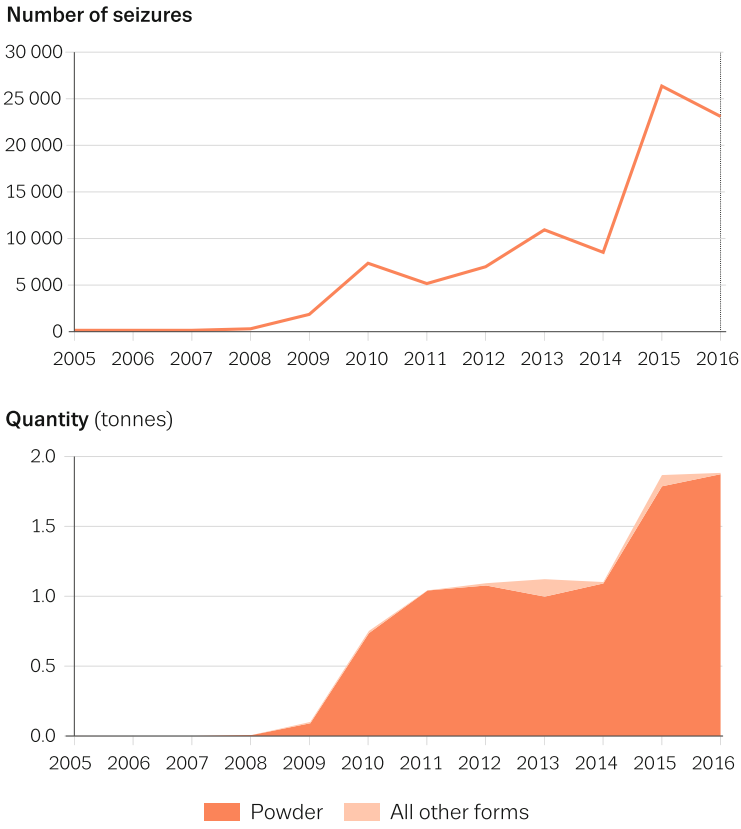


Fig. 5 Seizures by law enforcement of synthetic cathinones reported to the EU Early Warning System: trends in number of seizures and quantity seized, 2005–2016. © European Monitoring Centre for Drugs and Drug Addiction, 2018. Reproduction is authorized provided the source is acknowledged

as "not for human consumption" as well as "laboratory reagents." Some of the "dietary supplements" are advertised as "natural" products in order to avoid regulatory scrutiny as well as to dupe consumers into thinking that such products are safe and healthy options – a trick that is also widely used to sell unlicensed and fake medicines, particularly those for weight loss, sexual enhancement, and performance enhancement ("doping") (Evans-Brown et al. 2014; Abbate et al. 2015; Cohen et al. 2016). More recently, with vaping on the rise, e-liquids containing SCRAs and fentanils have appeared on the market, while the sale of ready-to-use nasal sprays containing fentanils has also increased in some areas (EMCDDA 2017a, 2018b; Peace et al. 2017; Helander et al. 2017; Ujváry et al. 2017; Södertörns Tingsrätt 2018) (Fig. 8).

Increasingly, NPS are also repackaged into smaller quantities or made into tablets and other dosage forms which are then sold on the illicit drug market either under

Fig. 6 A seizure of two packages each containing approximately 500 g of cyclopropylfentanyl. The seizure was made by the Polish Customs Service in September 2017. The packages were shipped from China and had transited through Belgium before being seized in Poland. Cyclopropylfentanyl is a derivative of fentanyl and was involved in more than 80 deaths in Europe during 2017. Images kindly provided by Central Customs and Tax Laboratory, Poland. © Central Customs and Tax Laboratory

their own name or passed off as established controlled drugs to unsuspecting users. New benzodiazepines and new synthetic opioids are also used to make fake tablets of commonly prescribed benzodiazepine and opioid analgesic medicines, these too are also sold on the illicit market (Fig. 9) (EMCDDA 2016a, 2018a, b). Sales are through existing street-level drug markets as well as online markets, including on the darknet (EMCDDA 2016b; National Crime Agency 2018).

3.2 "Spice," Smoking Mixtures, and the Synthetic Cannabinoid Receptor Agonists

One of the most popular types of "legal high" products over the last decade has been those sold as "legal" replacements to cannabis. They first began to appear in Europe around the mid-2000s as products called "Spice" but are known by many other names including "smoking mixtures," "herbal incense," "K2," "black mamba," and "fake weed" (EMCDDA 2009, 2017b; Jack 2009). Initially, Spice appeared to be a relatively harmless blend of plant material. It was advertised as a "exotic herbal blend" that "released a rich fragrance when burned" (Fig. 10). People who smoked

Fig. 7 Seizure of 40 kg of highly pure MDMB-CHMICA powder that was intercepted by Luxembourg Customs in December 2014. The powder was contained in forty 1 kg packages and was seized at Luxembourg Airport where it was in transit from China to Spain. The quantity seized would have been sufficient to make millions of doses as smoking mixtures. Images kindly provided by Luxembourg Customs. © Luxembourg Customs

Fig. 8 Unlabeled nasal sprays containing acryloylfentanyl that were sold online in Sweden in 2016. In the past few years, nasal sprays containing fentanils have become increasingly common in parts of Europe. Compared to injecting, nasal sprays make it easier for people to use fentanils while still giving them a similar psychoactive effect. Their use can pose a high risk of accidental poisoning. With their ease of use, nasal sprays could make the use of fentanils more attractive and socially acceptable, helping the use of these substances spread more widely. Image kindly provided by Prof. Anders Helander, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden. © Prof. Anders Helander

Spice claimed that it had "strong" cannabis-like effects, but it was not until 2008 that researchers discovered that in fact the plant material was laced with synthetic cannabinoid receptor agonists (SCRAs) such as JWH-018 and HU-210 (Auwärter et al. 2009; Griffiths et al. 2013; EMCDDA 2017b) – substances that mimic the effects of THC, which is the main psychoactive constituent of cannabis (Gaoni and Mechoulam 1964; Huestis et al. 2001). THC's effects on the central nervous system are believed to predominately involve activation of the CB_1 cannabinoid receptor that mediates the psychopharmacological effects (Gaoni and Mechoulam 1964; Huestis et al. 2001; Pertwee and Cascio 2014). Similar to THC, SCRAs also activate these receptors that form part of the endocannabinoid system – a system that helps regulate a large number of physiological functions in the body such as behavior, mood, pain, and appetite (Pertwee 2015). Many SCRAs were first developed to study this system and in the hope of developing new medicines (Pertwee 2005, 2015; Reggio 2009). Since 2008, 179 SCRAs have been detected on the drug market in hundreds of different products, making them the largest group of substances monitored by the EMCDDA. Alongside being sold as "legal" replacements to cannabis, some people also use them to avoid positive drug screens performed in the criminal justice system, in drug treatment programs, as well as in the workplace. Most smoking mixtures are made in Europe, sometimes on an industrial scale. The SCRAs are typically imported as powders from China (Fig. 7), dissolved using solvents such as acetone or methanol, and then mixed with or sprayed onto plant

Fig. 9 Fake Xanax benzodiazepine tablets containing cyclopropylfentanyl. The tablets were seized by Swedish Police in 2017. As users are unaware that such tablets contain highly potent opioids, they are at risk of life-threatening respiratory depression. A number of mass poisoning events have been reported in North America due to this type of adulteration. Those at particularly high risk include users who may not have any tolerance to opioids. Image kindly provided by Swedish Police. © Swedish Police

material such as *Turnera diffusa* (damiana) (Schäffer et al. 2013). Equipment such as cement mixers may be used to mix the ingredients together. The mixture is then dried and packaged (Fig. 11a, b) (EMCDDA 2017b).

Compared to cannabis, SCRAs can cause more profound physical and psychoactive effects (Ford et al. 2017; Zaurova et al. 2016). Severe and fatal poisoning is also more common (Tait et al. 2016; Winstock and Barratt 2013; Zaurova et al. 2016; EMCDDA 2018b). These include serious cardiovascular toxicity (including sudden death), rapid loss of consciousness/coma, respiratory depression, seizures and convulsions, hyperemesis, delirium, agitation, psychosis, and aggressive and violent behavior (Hill et al. 2016; Winstock and Barratt 2013; Tait et al. 2016; Zaurova et al. 2016; EMCDDA 2018b). There is no antidote to poisoning caused by SCRAs. The reasons behind these severe effects are not particularly well understood, but two important factors are the high potency of the SCRAs and the unintentionally high doses that users are exposed to.

Firstly, nonclinical studies have shown that many of the SCRAs sold on the drug market are much more potent than THC (behaving as so-called full agonists compared to THC that is a partial agonist). This means that even at very small doses, they can activate the CB_1 receptor much more strongly than THC (Banister et al. 2016; EMCDDA 2017c; Ford et al. 2017; Reggio 2009; Tai and Fantegrossi 2017; Wiley et al. 2017). Secondly, the process for mixing the SCRAs with the plant material to make smoking mixtures can lead to dangerous amounts of the substances in the products. This is because producers have to guess the amount of substances to be added, while the mixing process makes it difficult to dilute them sufficiently and distribute them consistently throughout the plant material. This can result both in products that contain toxic amounts of the substances in general (Ernst et al. 2017;

Fig. 10 "Spice" cigarette seized by Slovenian Police in 2009. The plant material in the cigarette contained CP-47,497 homolog C8 (bottom right-hand corner). Image kindly provided by Slovenian National Forensic Laboratory (Police). © Slovenian National Forensic Laboratory (Police)

Frinculescu et al. 2017; Langer et al. 2014, 2016) and in products where the SCRAs are clumped together, forming highly concentrated pockets within the plant material (Fig. 12) (Frinculescu et al. 2017; Moosmann et al. 2015; Schäper 2016). In fact, in the latter case, simply tapping a packet can dislodge the substances from the plant material. These issues are made worse because the products are smoked (or vaped), allowing the substances to be rapidly absorbed into the bloodstream and to reach the central nervous system and other parts of the body to cause their effects.

The combination of these two factors makes it difficult for users to control the dose that they are exposed to. This can lead them to unintentionally administer a toxic dose. Accounts from patients and people who witness poisonings suggest that in some cases a small number of puffs from a cigarette ("joint") have been sufficient to cause severe and fatal poisoning. In the last few years, there has been an increase in the number of deaths reported to the EMCDDA involving these substances (EMCDDA 2018b). These factors are also responsible for explosive outbreaks of mass poisonings caused by smoking mixtures containing SCRAs. These have ranged in size from a handful of victims to over 800 people, some of whom have died. Such outbreaks can also rapidly overwhelm the capacity of emergency responders and hospital emergency departments. Many of the outbreaks that have

Fig. 11 (**a**) "Legal high" smoking mixtures containing the synthetic cannabinoid receptor agonist CUMYL-PeGACLONE that were seized in Germany in 2016. Image kindly provided by Dr. Jan Schäper, Bayerisches Landeskriminalamt, Munich, Germany. © Dr. Jan Schäper. (**b**) Plain packages of smoking mixtures containing the synthetic cannabinoid receptor agonist CUMYL-PeGACLONE that were seized in Germany in 2016. Image kindly provided by Dr. Jan Schäper, Bayerisches Landeskriminalamt, Munich, Germany. © Dr. Jan Schäper

been reported are from the United States, but they have also occurred in Russia and Europe (Kasper et al. 2015; Shevyrin et al. 2015; Trecki et al. 2015; Adamowicz 2016; Pap 2018).

Fig. 12 Police seizure of "Green Hammock Potpourri" which was sold in Germany during 2014. The product contained MDMB-CHMICA, a synthetic cannabinoid receptor agonist that was linked to more than 28 deaths in Europe between 2014 and 2016. In the bottom left-hand corner of the image are large white lumps of MDMB-CHMICA that were found in the product. Due to the high potency of MDMB-CHMICA, such large amounts pose a risk of severe and even fatal poisoning to users. Originally published in Schäper (2016). Image kindly provided by Dr. Jan Schäper, Bayerisches Landeskriminalamt, Munich, Germany. © Dr. Jan Schäper

Some of the features of poisoning – particularly loss of consciousness, respiratory depression, and behavioral effects – may also place users at risk of choking on/aspirating vomit, drowning, falling, hypothermia as a result of falling unconscious outside in cold weather, and self-inflicted violence/injury (Tait et al. 2016; Yeter 2017; EMCDDA 2018b). The aggressive and violent behaviors reported with SCRAs may also place others at risk of injury (EMCDDA 2018b). Some people who suddenly stop using SCRAs after regular use have also reported withdrawal- and dependence-like symptoms, which can be severe (Macfarlane and Christie 2015; Cooper 2016).

While the number of SCRAs appearing for the first time each year is slowing down and there has been a drop in the amount of bulk powders seized in recent years, they continue to be available across much of Europe. In some areas, these substances are no longer just touted as "legal" replacements to cannabis but have developed a reputation among vulnerable groups, such as the homeless and prisoners, as powerful and cheap intoxicants that are used for their "mind-numbing" effects. Due to their high potency only small quantities are required, making them easier to smuggle into prisons; this includes impregnating letters and photographs with the substances (Ford and Berg 2018). The spread of these products has exacerbated existing health and social problems as well as created new ones for these groups. In prisons,

alongside the adverse health effects, the market in SCRAs has been linked to an increase in aggression, violence, bullying, and debt. In some cases this has caused a serious threat to the overall safety and security of the prison environment (Blackman and Bradley 2017; HMIP 2015; Ralphs et al. 2017; User Voice 2016).

Since 2016, the EMCDDA has conducted five joint investigations with Europol (Sect. 4.3.2) on synthetic cannabinoids that have caused serious concern at European level. These are MDMB-CHMICA in 2016 and AB-CHMINACA, ADB-CHMINACA, 5F-MDMB-PINACA, and CUMYL-4CN-BINACA during 2017. Together, these substances have been involved in more than 100 deaths, many of which were attributed directly to these substances. All five of these substances were formally risk assessed by the EMCDDA (Sect. 4.3.3) during 2016–2017.

3.3 New Synthetic Opioids and the Fentanils

Over the past few years, there has been a large increase in the availability of new synthetic opioids in Europe (EMCDDA 2018b). This has been driven by interest in selling these substances in Europe but also possibly due to a general increase in availability that is linked to the ongoing opioid epidemics in the United States and Canada (US CDC 2015; Gladden et al. 2016; O'Donnell et al. 2017).

Since 2009, a total of 38 new opioids have been detected in Europe; this includes 22 that were reported for the first time between 2016 and 2017. While currently playing a small role in the overall market, new opioids are of special concern to public health because they can pose a high risk of life-threatening poisoning from respiratory depression (Cox 2011; Dahan et al. 2010; Kieffer 1999; Pattinson 2008; Romberg et al. 2003; White and Irvine 1999). Most of the opioids that have been reported so far are derivatives and analogs of the analgesic fentanyl.

During the 1960s, attempts to develop better and safer analgesic medicines led to the synthesis and testing of a series of new opioid narcotic analgesic drugs by the pharmaceutical company Janssen Pharmaceutica. Fentanyl was the first substance in this highly potent family to be synthesized and was followed by a series of related substances, which together are known as the fentanils (Janssen 1982; Janssen and Van der Eycken 1968). Since then, dozens more of these substances have been synthesized and tested by scientists. A small number – fentanyl, alfentanil, sufentanil, and remifentanil – have become widely used in human medicine in anesthesia and for pain management, while some are used in veterinary medicine in anesthesia and for pain management and, in the case of carfentanil and thiafentanil, to immobilize large animals. Similar to the SCRAs, some of the fentanils are also used to study how the body works, provide insights into disease, and help develop new medicines (Ujváry et al. 2017).

Alongside their legitimate uses as medicines and in research and as highlighted in Sect. 2, the fentanils also have a long history of illicit use as replacements for heroin and other controlled opioids. Between 1979 and 1988, more than ten fentanils that had been made in illicit laboratories were detected on the drug market in the United

States (Henderson 1991). The first was α-methylfentanyl (Kram et al. 1981), followed by substances such as 3-methylfentanyl and 4-fluorofentanyl. Typically, they were sold as heroin or "synthetic heroin." Together, these substances were involved in more than 100 deaths, mostly in the state of California. Later, in the mid-2000s, illicitly manufactured fentanyl was sold as heroin or in mixtures with heroin and was responsible for outbreaks of overdoses that involved hundreds of deaths in the Eastern United States (Schumann et al. 2008). It appears that, with the exception of Estonia, where 3-methylfentanyl and fentanyl were responsible for an epidemic of fatal poisonings during this time, these substances caused limited problems elsewhere in Europe (Sorokin et al. 1994; Sorokin 1994; Berens et al. 1996; de Boer et al. 2003; Fritschi and Klein 1995; Kronstrand et al. 1997; Ojanperä et al. 2006, 2008; Poortman-van der Meer and Huizer 1996).

However, since 2012 the situation has changed dramatically. So far, 28 new fentanils have been reported, including 18 between 2016 and 2017. While the picture differs widely across Europe, 23 countries have reported detections of one or more of the substances (Fig. 13). There has also been a large increase in seizures reported by customs at international borders and by police at street level in recent years (Fig. 14).

Where known, most shipments of new fentanils coming into Europe originate from companies based in China. Production in illicit laboratories, including in Europe, has also been reported occasionally. Typically, production of fentanyl and other fentanils is relatively straightforward, which adds to the challenges in responding to these substances.

These substances are sold and used as "legal" substitutes for illicit opioids and prescription opioid medicines; this may include for self-medication, such as treating pain and/or opioid withdrawal. In addition, they are also sold as or in mixtures with heroin and other illicit opioids (EMCDDA 2017d, 2018b). Information from law enforcement and death investigations in Europe have found that they are used by vulnerable and marginalized opioid users, including those who inject heroin and other illicit opioids (Guerrieri et al. 2017a, b; Elliott and Hernandez Lopez 2018; Hikin et al. 2018).

Fentanils have been found in a variety of physical and dosage forms in Europe. They are most commonly encountered as powders, but they have also been detected in liquids and tablets. Depending on the circumstances, seizures of powders have ranged from milligram to kilogram quantities (Figs. 6 and 15). They may be relatively pure, especially when seized coming into the European Union. They may also be mixed with one or more substances. In the latter case, these include commonly used cutting agents (such as mannitol, lactose, and paracetamol), as well as heroin and other fentanils/opioids. To a much smaller degree, other drugs, such as cocaine and other stimulants, have also been detected in mixtures with fentanils in Europe. During 2016, more than 4.6 kg of powder-containing fentanils was reported, while almost 4.5 L of liquid and around 2,900 tablets were also reported (Fig. 14). Less commonly, fentanils have also been found in blotters and plant material; in these cases, there may be no indication that they contain fentanils, which could pose a risk of poisoning to people who use them.

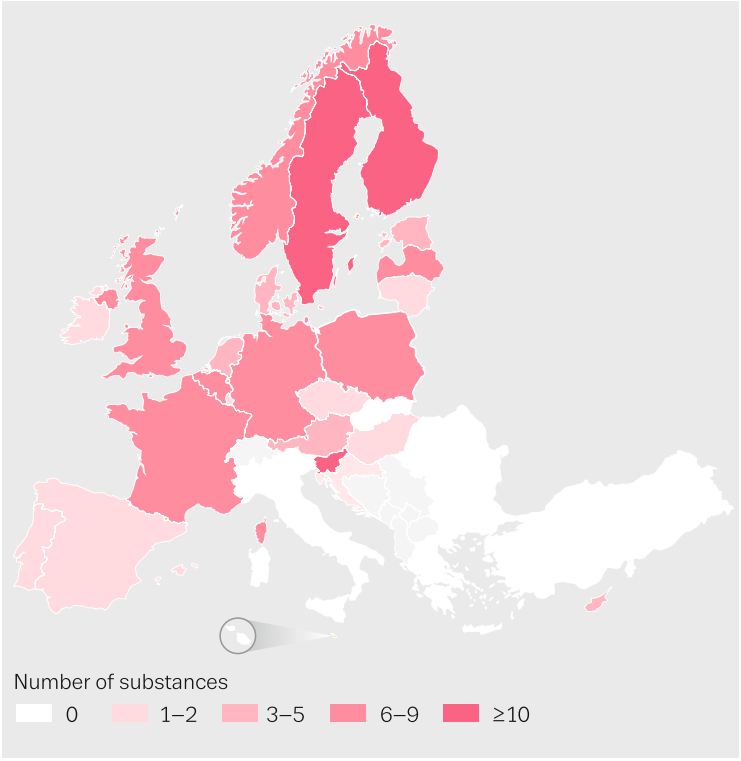


Fig. 13 Number of different fentanils detected by country reported to the EU Early Warning System, 2012–2017. Note that a "detection" may include any sample that is analytically confirmed and that is from a seizure made by law enforcement or a collected sample that are actively collected by drug monitoring systems (such as test purchases or drug testing programs) or from human biological samples. © European Monitoring Centre for Drugs and Drug Addiction, 2018. Reproduction is authorized provided the source is acknowledged

Like other types of opioid analgesics, such as morphine and heroin, the fentanils produce their main effects by activating the μ-opioid receptor in the central nervous system (Cox 2011; Pasternak and Pan 2013; Ujváry et al. 2017). These include euphoria, relaxation, analgesia, sedation, bradycardia, hypothermia, and respiratory depression (Cox 2011; Dahan et al. 2001; Kieffer 1999; Pattinson 2008; Romberg et al. 2003). It is this last effect that poses the greatest danger to users, as, because of the high potency of these substances, small amounts can cause severe, life-threatening poisoning from respiratory depression. Left untreated, this can be fatal (Cox 2011; Dahan et al. 2010; Pattinson 2008; Somerville et al. 2017; White and Irvine 1999). Fentanils also have an abuse liability and dependence potential.

While fentanils are often injected intravenously, their high potency and ease of use mean that nasal sprays containing diluted solutions have become an increasingly common way of using these substances in some parts of Europe. Unlabeled nasal

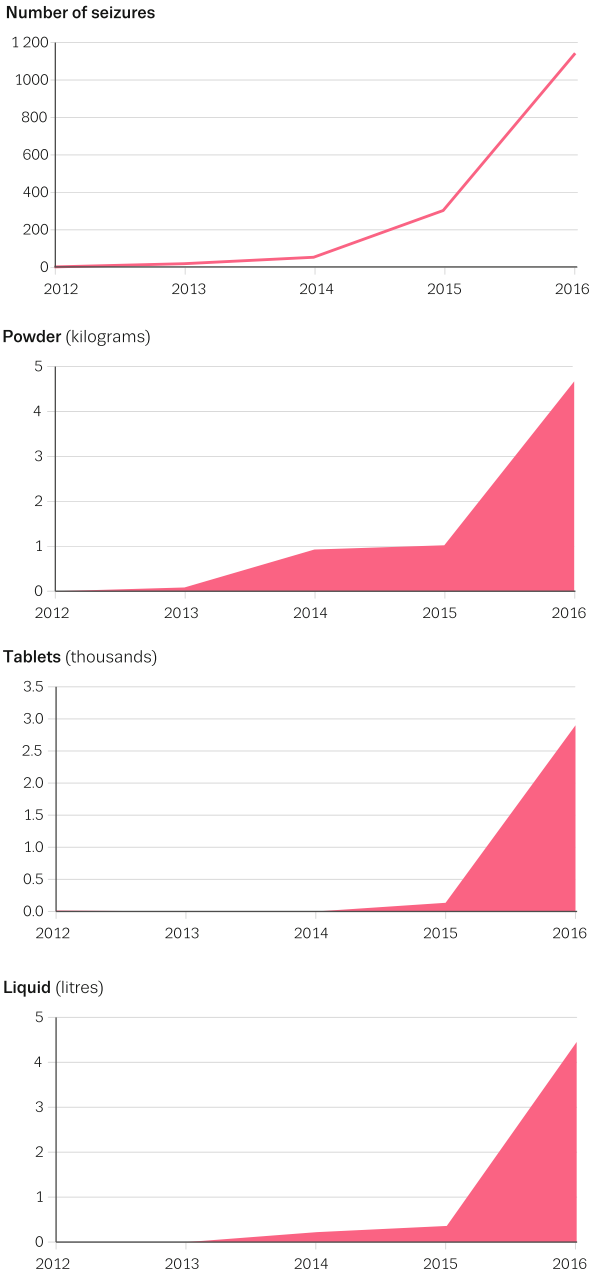


Fig. 14 Seizures of fentanils reported to the EU Early Warning System: trends in number of seizures and quantity seized, 2012–2016. © European Monitoring Centre for Drugs and Drug Addiction, 2018. Reproduction is authorized provided the source is acknowledged

sprays containing acryloylfentanyl, offered for sale online, were detected in Sweden in 2016 (Fig. 8). This substance was involved in 47 deaths in Europe during 2016 (EMCDDA 2017a; Guerrieri et al. 2017b; Helander et al. 2017; Ujváry et al. 2017;

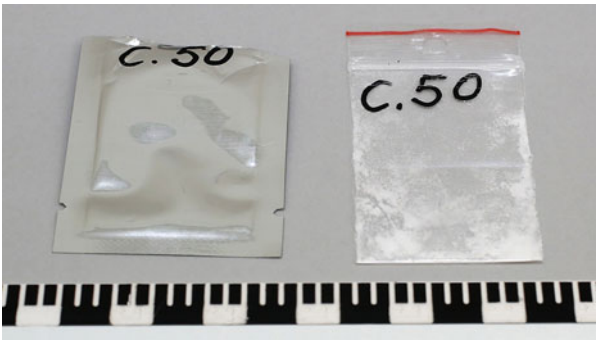


Fig. 15 Foil packet containing a ziplock bag labeled "C.50" that contained carfentanil as a white powder. It was bought on a darknet market and recovered from a scene of death in Norway in 2017. Between November 2016 and April 2017, carfentanil was involved in at least 61 deaths in 8 countries in Europe. In many cases, those who died were people who used heroin. Image kindly provided by Norwegian National Criminal Investigation Service (Kripos). © Norwegian National Criminal Investigation Service (Kripos)

Södertörns Tingsrätt 2018). E-liquids containing fentanils that can be vaped using electronic cigarettes have also been reported. Compared with injecting, these dosage forms make it easier for people to use fentanils while still giving them a similar psychoactive effect (e.g., Macleod et al. 2012). Their use may also pose a high risk of accidental overdose. Nasal sprays and e-liquids could make using fentanils more attractive and socially acceptable, helping them spread more widely.

The risk of poisoning by fentanils may be exacerbated by a number of factors including the difficulty in diluting fentanils because of their high potency, which can lead to a toxic dose being accidentally used; using routes of administration that allow large amounts of the substance to rapidly reach the central nervous system (such as injecting, insufflation, and inhalation); availability of easy to use dosage forms (such as nasal sprays and e-liquids); lack of awareness and experience of users with these new substances (effects and dosage); use of other central nervous system depressants (such as other opioids, benzodiazepines, and alcohol); lack of tolerance to opioids in new or former users; use in environments where it may be difficult to summon help in the event of poisoning (e.g., alone in a home environment); and limited availability of the antidote naloxone (EMCDDA 2016c).

Timely administration of naloxone can rapidly reverse the severe respiratory depression caused by fentanils (Kim and Nelson 2015). Of note is that recent experiences in the United States and Canada suggest that, compared with treating heroin overdoses, larger and additional doses of naloxone have been required in some cases in order to reverse the respiratory depression (Faul et al. 2017; Klar et al. 2016; Somerville et al. 2017; Sutter et al. 2017). While this finding needs further study, it could be due to factors such as the high potency of the fentanils, the dose an individual is exposed to, and the relatively short half-life of naloxone. Patients may need longer periods of observation after initial treatment in case respiratory depression reoccurs (Sutter et al. 2017; Uddayasankar et al. 2018).

Since late 2015, the EMCDDA has conducted eight joint investigations with Europol (Sect. 4.3.2) on fentanils that have caused serious concern at European level. The two agencies investigated acetylfentanyl in 2015, acryloylfentanyl and furanylfentanyl in 2016, and 4-fluoroisobutyrylfentanyl (4F-iBF), tetrahydrofuranylfentanyl (THF-F), carfentanil, methoxyacetylfentanyl, and cyclopropylfentanyl during 2017. Together, these substances have been involved in more than 250 deaths, many of which were attributed directly to these substances. Five of these substances were formally risk assessed by the EMCDDA (Sect. 4.3.3) during 2017, and methoxyacetylfentanyl and cyclopropylfentanyl were assessed early in 2018.

3.4 Recent Developments

In the past few years, there have been some major developments in the market in Europe. Some of these are encouraging. The number of new substances detected for the first time each year during 2016–2017 has fallen by around 40% compared to 2015. Much of this is related to a decrease in the number of new SCRAs and synthetic cathinones appearing each year. There has also been a fall in the number of seizures involving SCRAs in powder form, which may indicate a decrease in the production of smoking mixtures. In part, this may reflect the results of sustained efforts to control new substances in Europe, including their open sale as "legal highs" on the high street. Law enforcement operations in China leading to the closure of companies making these substances might be another reason (EMCDDA 2018a, b).

Other developments are concerning. Overall, the availability of many new substances remains relatively high, and, in places, strong links exist with established illicit drug market (EMCDDA 2018b); in the latter case this includes the use of new benzodiazepines to make fake tablets of commonly prescribed benzodiazepine medicines such as diazepam and alprazolam. In Europe, it appears that there is increasing interest from crime groups in making new substances, such as synthetic cathinones (EMCDDA 2016a). Globalized markets, where new psychoactive substances and other ingredients can be produced in one country, brokered and used in production in others, and finally used in still others, increase the opportunity for miscommunication, substitution, mislabeling, and adulteration. The sheer number and quantity of substances currently on the market – approximately half of 670 substances currently being monitored were detected in Europe during 2016 – increase the risk of them being sold either deliberately or accidentally as other drugs, which can lead to life-threatening poisoning that can manifest as outbreaks (e.g., Pap 2016; Quintana et al. 2017; EMCDDA 2018b; Horth et al. 2018). An important example here can be found in the fentanils, which have been sold as heroin and, less commonly, as fake benzodiazepine and opioid analgesic medicines, and also cocaine. Such scenarios can pose a high risk of life-threatening respiratory depression in users, especially those who have no tolerance to opioids (SFDPH 2015; Klar et al. 2016; Tomassoni et al. 2017; Sutter et al. 2017).

Another major and related challenge in the last few years has been the large number of highly potent substances that have appeared on the market. As highlighted above, this includes the SCRAs (Sect. 3.2) and the fentanils (Sect. 3.3) (EMCDDA 2017c, d). Such substances are easier to conceal and smuggle, with a few grams sufficient to make many thousands of doses for the drug market. This makes them easier to import undetected into Europe and, from there, move across the rest of Europe, as small letters and packages can easily be disguised. As the volume of express mail, courier, and air and sea cargo increases, the detection of new substances is likely to cause significant challenges to customs and border agencies. These substances also pose a high risk of life-threatening poisoning to users and are capable of causing explosive outbreaks that can overwhelm local healthcare systems. While formal estimates are lacking, responding to such outbreaks is also invariably financially costly. In addition, in some circumstances, law enforcement, first responders, and laboratory personnel may be at risk of poisoning from occupational exposure (Dobaja et al. 2017; Tapp et al. 2017; US IABESI 2017; US WHNSC 2017; EMCDDA 2018b). Given the globalized nature of the market, these types of substances can pose a serious cross-border threat to health (Decision No 1082/2013/EU 2013; WHO 2015; Regulation (EU) 2017/2101).

4 Responding to New Psychoactive Substances in the European Union

4.1 Legal Framework

For more than 20 years, a specific legal framework has been in place in the European Union that allows it to respond to the appearance of new psychoactive substances on the drug market. The origins of this legislation lie in the surprise appearance and popularity of MDMA and other similar synthetic drugs in Europe, often as a part of the acid house, electronic dance music, and rave scenes (Collin 1997; Reynolds 1999). As demand rose, some of these drugs were produced on a relatively large scale in illicit laboratories run by organized crime groups. As a result, governments identified the need to share information on these types of substances as well as strengthen law enforcement and judicial cooperation (96/750/JHA 1996). In 1997 this led to the introduction of the first piece of legislation known as the *Joint Action on New Synthetic Drugs* (97/396/JHA 1997). The legislation defined a three-step process of information exchange (commonly known as "early warning"), risk assessment, and control measures. Together, this framework allowed Europe to detect, assess, and respond to public health and social threats caused by such substances.

In 2005, the Joint Action was replaced by the *Council Decision 2005/387/JHA*, which kept the three-step approach but extended the scope and strengthened the overall system (Proposal for a Council Decision 2003; Council Decision 2005/387/JHA). The term "new psychoactive substances" was also used for the first time and given legal meaning, being defined as substances not currently listed in any of the

schedules to the United Nations Single Convention on Narcotic Drugs (1961), as amended by the 1972 Protocol, that may pose a comparable threat as the substances listed in Schedules I or II or IV thereof and the United Nations Convention on Psychotropic Substances (1971) that may pose a comparable threat to public health as the substances listed in Schedule I or II or III or IV thereof (Council Decision 2005/387/JHA 2005; Regulation (EC) No 1920/2006).

In response to the recent growth in the market in new substances, the *Council Decision* will be replaced in 2018 with new legislation that retains the three-step approach while strengthening early warning and risk assessment procedures as well as introducing shorter deadlines to each step. The legislation will apply from 23 November 2018 (Regulation (EU) 2017/2101; Directive (EU) 2017/2103).

4.2 European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)

The EMCDDA is an agency of the European Union. It was established in 1993 in the face of a growing drug problem in Europe and based on the premise that independent, science-based information is an essential resource to help Europe understand the nature of its drug problems and better respond to them (EMCDDA 2018c). The objective of the EMCDDA is to provide the European Union and its member states with factual, objective, reliable, and comparable information at European level concerning drugs and drug addiction and their consequences (Estievenart 1995; Regulation (EC) No 1920/2006).

The EMCDDA provides policymakers with the data needed for developing informed drug laws and strategies. It also supports professionals and practitioners working in the field to identify best practice and new areas of research. To achieve its task of providing sound and comparable information on drugs in Europe, the EMCDDA has developed an infrastructure and tools to collect country data in a harmonized way. These data are then provided by national drug monitoring centers, known as the Reitox network, to the EMCDDA for analysis, resulting in a variety of information products conveying the broader European picture (EMCDDA 2018d).

In addition to these tasks, the EMCDDA has also played a central role in the EU's response to NPS since the first piece of legislation came into force in 1997, being responsible for operating an early warning system with Europol and conducting risk assessments (Council Decision 2005/387/JHA 2005; Regulation (EC) No 1920/2006; EMCDDA 2018e).

4.3 Early Warning

4.3.1 European Union Early Warning System

The early warning step of the Council Decision is known as the *European Union Early Warning System on New Psychoactive Substances* (EU Early Warning

System, EWS) (Council Decision 2005/387/JHA 2005; Regulation (EC) No 1920/2006). The system is composed of a multidisciplinary and multiagency network, which includes the EMCDDA, 30 national early warning systems (28 member states, Turkey, and Norway), Europol and its law enforcement networks, the European Medicine Agency, the European Commission, and other partners (EMCDDA 2018e). The EMCDDA is responsible for collecting, collating, analyzing, assessing, and communicating the information reported by the network. In the latter case, this is done both by email and the EMCDDA's dedicated information system.

Underpinning each of the national systems, and, in turn, the EU EWS, is the dissemination of data on the chemical identification of new substances from forensic and toxicology laboratories. Principally, these laboratories handle casework related to seizures of NPS by law enforcement agencies (including shipments detained for inspection that are made by customs or border agencies) and from nonfatal and fatal poisonings (such as those from hospital emergency departments and medicolegal death investigations). Overall, such an approach allows the collection and rapid reporting of event-based information on the appearance of, and harms caused by, NPS at national level to the EMCDDA. These data are complemented by biannual reports, which include aggregated data on seizures by law enforcement and from poisonings. The organization and functioning of the national early warning systems is a national responsibility. While these systems have developed to meet national needs, they draw on a common format and guidelines to report information to the EMCDDA (Council Decision 2005/387/JHA 2005; EMCDDA 2007).

Most NPS are identified for the first time following the chemical analysis of a seizure made by law enforcement. When a substance is suspected of being a NPS, the national early warning system reports this to the EMCDDA. This includes chemical and analytical information, as well as the circumstances of the event. The submission of analytical data is also required; to a certain extent, such data are substitutes for reference standards, which are often not available when a substance is first detected (EMCDDA 2007).

Following a review of the reported information, the EMCDDA identify other relevant information that may be found in the literature. If confirmed as a new substance, then a formal notification is issued on behalf of the reporting country. The notification includes the names and identifiers of the substance, chemical and physical properties, analytical methodologies for its identification, pharmacology, toxicology, circumstances of the detection, and any other relevant information. At this stage, the EMCDDA begins to formally monitor the substance. The notification process is one of the cornerstones of a successful early warning system as it strengthens situational awareness, preparedness, and responses. By ensuring that members of the network are alerted as soon as possible to the detection of a new substance in Europe, it allows analysis of any potential threats, as well as the forensic and toxicology laboratories, to begin the process of including the substance in their analytical screening allowing it to be detected and therefore monitored. In addition, availability of new information might allow analytical laboratories to retrospectively

analyze their data for the identification of NPS that might have been missed, for example, during targeted screening procedures.

The rapidly changing nature of the NPS market, its links with the established illicit market, and the overall large number of substances that need to be monitored have presented challenges for early warning activities in recent years. In response to this, the EMCDDA has undertaken a program of work to strengthen early warning activities. This includes developing a range of interconnected systems as part of the EU EWS – including a toxicovigilance system, signal management system, open-source information monitoring system, and risk communication system – that allows it to better detect, assess, prioritize, and respond to potential and identified public health and social threats associated with NPS.

The toxicovigilance system allows the EMCDDA to detect, assess, and react to serious adverse events associated with new substances. A particular focus of this work has involved harmonizing the way information on acute nonfatal and fatal poisonings is reported and managed. The signal management system provides a framework to detect, assess, and prioritize threats associated with new substances. Related to this, the EMCDDA has also been developing a system to monitor open-source information (Olcott 2012; Hartley et al. 2013; Dion et al. 2015) that improves both general situational awareness (Olcott 2012; Dekker 2014) and the capacity to detect signals of serious and urgent health threats that are of relevance to the European Union. In part, this is increasingly important both due to globalized supply chains for NPS (and other ingredients used in such products) and because of a growing number of outbreaks of mass poisonings linked to NPS. This multilingual system includes the use of the medical information system (MedISys), which is developed by the European Commission's Joint Research Centre (Linge et al. 2012; Mantero et al. 2014), Google Alerts, Twitter, as well as other sources, and monitors events from thousands of sources of information, such as the media, health agencies, and law enforcement. Important types of events detected by this system include outbreaks of mass poisonings caused by new substances. This includes those linked to SCRAs, such as in Russia when MDMB-FUBINACA was involved in 600 poisonings over a 2-week period (Shevyrin et al. 2015), a multistate outbreak in the United States involving ADB-CHMINACA (Kasper et al. 2015), and, more recently, a multistate outbreak of severe bleeding that poisoned more than 200 people caused by smoking mixtures containing the anticoagulant rodenticide brodifacoum (US CDC 2018; Moritz et al. 2018). In response to the recent appearance of large numbers of fentanils, the EMCDDA has also developed a specific data feed for monitoring events linked to this highly dangerous group of substances. Finally, work has also been done on strengthening risk communication to the network related to important signals and threats that the EMCDDA identifies through its early warning and risk assessment activities.

4.3.2 Joint Report

If the EMCDDA and Europol or the Council consider that the information collected on a new substance reported by the network requires a formal response, then the two agencies undertake an investigation into the substance. Known as a *Joint Report*, the

analysis provided is used by the Commission and the Council to determine if a formal risk assessment is required. This marks the final stage of early warning (Council Decision 2005/387/JHA 2005; EMCDDA 2007).

4.3.3 Risk Assessment

Based on the analysis in the Joint Report, if the Council decides that a formal risk assessment of a new substance is required, then the Scientific Committee of the EMCDDA conducts a risk assessment. The EMCDDA acts as the secretariat for the assessment. Guidelines provide a methodological and procedural basis for carrying out a risk assessment, including providing a conceptual framework for consideration of risk. The risk assessment component is an important step to support decision-making on new psychoactive substances at EU level, adding value to national actions in this area (Council Decision 2005/387/JHA; Regulation (EC) No 1920/2006; EMCDDA 2010).

Using data reported by the network and identified by the EMCDDA through its other monitoring systems, the risk assessment process reviews the possible health and social risks of the substance and the implications of placing it under control. In general, the scientific knowledge on a NPS will accumulate over time and as experience with the substance develops. In the interim, risk assessments are based on a broad range of available evidence, the quality of which needs to be appraised. Data reliability and relevance need to be assessed and weighed separately. For example, unpublished recent data may be considered to have a lower formal quality but still may be considered relevant. An important part of the assessment involves an analysis of the possible nature and risks of the substance with reference to similar known substances. This is important because, in part, data on the effects of new substances are often extremely limited. Such comparisons need not be restricted to controlled drugs but may include other substances with similar chemical characteristics, pharmacological actions, and psychological and behavioral effects or which otherwise offer relevant insights into the possible risks of the substance (EMCDDA 2010).

An assessment of the risk–benefit ratio of a NPS is also needed. Factors that may need to be considered include whether the substance has legitimate uses (e.g., potential therapeutic benefits), industrial use, or other economic value. Indeed, substances with a known therapeutic value or those that are used to manufacture medicinal products may be exempted from risk assessment. At the risk assessment stage, the prevalence of use of a new substance will usually be low. Here the majority of the available information comes from forensic and toxicology laboratories, law enforcement agencies, and anecdotal reports. Especially important here are reports relating to cases of nonfatal and fatal poisonings in which exposure to the NPS under assessment has been confirmed by chemical analysis of biological samples taken from cases (EMCDDA 2010).

The concept of risk includes both the element of probability that some harm may occur (usually defined as "risk") and the degree of seriousness of such a harm (usually defined as "hazard"). Substance-related risks can originate from several sources, and it is essential to clarify their type and origin. The risk assessment

conceptual framework differentiates between (a) sources from which substance hazards emanate and (b) types of hazardous effects that may be caused by substance use (EMCDDA 2010).

On completion of the risk assessment, a report is drawn up by the Scientific Committee containing an analysis of the scientific and law enforcement information available, reflecting all opinions held by the members of the Committee (Council Decision 2005/387/JHA 2005). The report includes:

- The physical and chemical description of the NPS and its mechanisms of action, including its medical value
- The health risks associated with the NPS
- The social risks associated with the NPS
- Information on the level of involvement of organized crime and information on seizures and/or detections by the authorities and the manufacture of the NPS
- Information on any assessment of the new psychoactive substance in the United Nations system
- Where appropriate, a description of the control measures that are applicable to the NPS in the member states
- Options for control and the possible consequences of the control measures
- The chemical precursors that are used for the manufacture of the substance

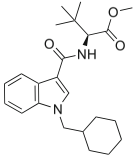
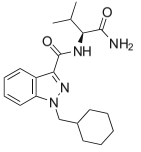
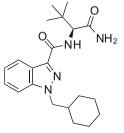
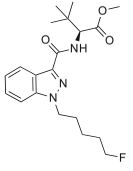
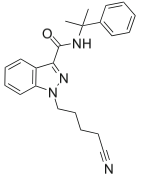
Since 1997, the EMCDDA has conducted 32 risk assessments on NPS. More than half of these have been conducted in the past 5 years, which reflects the growth in the market and an increase in harms reported in recent years (EMCDDA 2018e).

Key data from the risk assessments of five SCRAs (MDMB-CHMICA, AB-CHMINACA, ADB-CHMINACA, 5F-MDMB-PINACA, and CUMYL-4CN-BINACA) and five fentanils (acryloylfentanyl, furanylfentanyl, 4F-iBF, THF-F, and carfentanil) conducted between 2016 and 2018 are presented in Tables 1 and 2, respectively (EMCDDA 2018b).

4.3.4 Control Measures

The third and final step of the Council Decision is to consider the introduction of control measures. Following submission of a risk assessment report, the Council may decide to submit the NPS to control measures. Should this be the case, then the member states have to take the necessary control measures and criminal penalties, within 1 year from the date of the decision in accordance with their national law, and to submit as provided under their legislation by virtue of their obligations under the Single Convention on Narcotic Drugs (1961) or Convention on Psychotropic Substances (1971) (Council Decision 2005/387/JHA 2005).

Table 1 Key findings of the risk assessments of MDMB-CHMICA, AB-CHMINACA, ADB-CHMINACA, 5F-MDMB-PINACA, and CUMYL-4CN-BINACA conducted by the EMCDDA

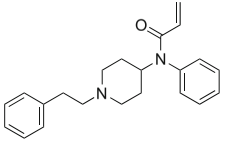
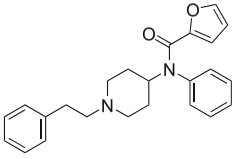
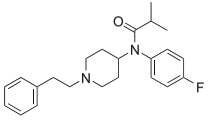
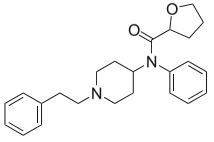
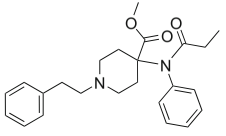
Common name	MDMB-CHMICA	AB-CHMINACA	ADB-CHMINACA	5F-MDMB-PINACA	CUMYL-4CN-BINACA
Chemical name	Methyl 2-[[1-(Cyclohexylmethyl)-1*H*-indole-3-carbonyl] amino]-3,3-dimethylbutanoate	*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide	*N*-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide	Methyl 2-{[1-(5-Fluorophenyl)-1*H*-indazole-3-carbonyl] amino}-3,3-dimethylbutanoate	1-(4-Cyanobutyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
Chemical structure					
Pharmacology	Full agonist at the CB_1 receptor; agonist at the CB_2 receptor	Full agonist at the CB_1 receptor; partial agonist at the CB_2 receptor	Full agonist at the CB_1 receptor; agonist at the CB_2 receptor	Full agonist at the CB_1 and CB_2 receptor	Full agonist at the CB_1 and CB_2 receptor
Formal notification to EU EWS	12 September 2014, Hungary	10 April 2014, Latvia	12 September 2014, Hungary	08 January 2015, Hungary	04 March 2016, Hungary
Number of deaths	29	31	13	28	11
Number of countries where deaths occurred	6	6	3	2	2
Number of seizures	>3,600	6,422	3,794	1986	2,461

(continued)

Table 1 (continued)

Common name	MDMB-CHMICA	AB-CHMINACA	ADB-CHMINACA	5F-MDMB-PINACA	CUMYL-4CN-BINACA
Number of countries where it has been seized	25	26	19	27	12
Total quantity seized	67 kg plant material 46 kg powder	190 kg plant material 44 kg powder 293 mL liquid 194 g blotters	139 kg plant material 10 kg powder 26 g blotters	100 kg plant material 13 kg powder 309 g and 94 mL liquid Blotters	261 kg plant material 52 kg powder Blotters

Table 2 Key findings of the risk assessments of acryloylfentanyl, furanylfentanyl, 4-fluoroisobutyrylfentanyl (4F-iBF), tetrahydrofuranylfentanyl (THF-F), and carfentanil conducted by the EMCDDA

Common name	Acryloylfentanyl	Furanylfentanyl	4F-iBF	THF-F	Carfentanil
Chemical name	*N*-(1-Phenethylpiperidin-4-yl)-*N*-phenylacrylamide	*N*-Phenyl-*N*-[1-(2-phenylethyl) piperidin-4-yl]furan-2-carboxamide	*N*-(4-Fluorophenyl)-*N*-(1-phenethylpiperidin-4-yl)isobutyramide	*N*-Phenyl-N-[1-(2-Phenylethyl) piperidin-4-yl] oxolane-2-carboxamide	Methyl 1-(2-phenylethyl)-4-[phenyl(propanoyl) amino]piperidine-4-carboxylate
Chemical structure					
Pharmacology	μ-Opioid receptor agonist	μ-Opioid receptor agonist	μ-Opioid receptor agonist	μ-Opioid receptor agonist	μ-Opioid receptor agonist
Formal notification to EU EWS	7 July 2016, Denmark	3 November 2015, Finland	26 August 2016, Slovenia	23 December 2016, Sweden	12 February 2013, Latvia
Number of deaths	47	23	20	14	61
Number of countries where associated deaths occurred	3	6	2	1	8
Number of seizures	162	143	24	53	801

(continued)

Table 2 (continued)

Common name	Acryloylfentanyl	Furanylfentanyl	4F-iBF	THF-F	Carfentanil
Number of countries where it has been seized	5	14	4	1	7
Total quantity seized	113 g powder 1,495 mL liquid 896 tablets	1,036 g powder 1,559 mL liquid 45 tablets 6 g plant material	379 g powder 208 mL liquid 6,727 tablets	99 g powder 950 mL liquid	3.3 kg powder

5 Conclusions

5.1 What Is Next for New Psychoactive Substances?

Humans have used drugs for thousands of years. Similar to other social and cultural phenomena, their use continues to change and evolve, being shaped by fads and fashions, new substances and products, new ways of taking drugs, new groups of users, globalization, new technologies, as well as the effects of policies and measures to regulate and prohibit. The large increase in NPS in Europe over the last decade is an important example of such changes and just how quickly new threats to health can emerge and establish themselves.

Yet, continued growth of this market at a similar pace to that seen over the last decade is not inevitable. There have been some encouraging signs, such as the recent 40% drop in the number of new substances reported for the first time during 2016–2017. This trend appears to be continuing, with 23 substances reported during the first 6 months of 2018. As noted, in part, this may reflect the results of sustained efforts to control new substances in Europe, including their open sale as "legal highs" on the high street, which will have a knock-on effect on the demand from retailers. While analysis of this issue is limited, it has been suggested that these approaches may have led producers and retailers in Europe to drop out of the market. Like most phenomena, part of the market is likely to have been driven by fads, with enthusiasm waning over time. It is possible that some individuals became involved in the market only because products were widely available and they saw an opportunity to make money quickly and easily. These suggestions are certainly worth further study in order to better understand how the market responds to different types of regulation and enforcement activities.

Nonetheless, major new challenges have also emerged. These include an increase in the number of highly potent new substances on the market, such as the SCRAs and fentanils. These types of substances pose a high risk of life-threatening poisoning to users and are capable of causing explosive outbreaks that can overwhelm local healthcare systems. While the picture differs greatly across Europe, the past few years has shown that it is not immune to such outbreaks. These substances are also easier to conceal and smuggle, with a few grams sufficient to make many thousands of doses for the drug market. It is also concerning that SCRAs have come to be used as powerful and cheap intoxicants because of their "mind-numbing" effects. Globalized markets also increase the opportunity for NPS (and a whole range of toxic chemicals) to be sold either deliberately or accidentally as other drugs, which can have disastrous consequences (Pap 2016; Klar et al. 2016; Sutter et al. 2017; EMCDDA 2018b). Strong links also exist between the trade in NPS and markets in established controlled drugs, with the increasing use of new benzodiazepines to make fake diazepam and alprazolam providing some indication of this.

Continued availability of NPS is driving greater complexity into the drug situation. Yet, what happens next is not easy to predict with certainty. Reflecting the globalized nature of the market, it is unclear what the impact will be on the availability of fentanils in Europe as a result of the recent generic control measures

introduced in the United States in February 2018 (US DOJ 2018). Other developments closer to home may also have an impact on the availability of these highly potent substances (National Crime Agency 2018; Södertörns Tingsrätt 2018). Nor is it yet clear what, if anything, might replace the fentanils as a result. Does a fall in reports of seizures of bulk powders of SCRAs, which are used to make smoking mixtures, perhaps indicate an important decline in production of these products in Europe?

As more and more people look to improve their health, well-being, and performance, what part will NPS play in the growing market in enhancement drugs (Evans-Brown et al. 2009; Savulescu et al. 2011)? These drugs include the so-called nootropics (not all of which are psychoactive) that are often sold under the guise of "dietary supplements" – adrafinil – to "boost concentration, wakefulness, and focus," while phenibut and tianeptine are sold to "brighten mood, increase confidence, and reduce anxiety" (Owen et al. 2016; Dempsey et al. 2017; Samuels et al. 2017; Bakota et al. 2018; Marraffa et al. 2018). Typically such substances are blind spots for early warning systems (Griffiths et al. 2013).

Globalized markets also allow existing regulatory systems, such as those for medicines, to be sidestepped more easily than in the past. Will people who are disillusioned with existing medical treatments (or lack thereof) go straight to online suppliers of NPS? Will the increasing regulation of psychoactive medicines drive more people to the illicit market (Martin et al. 2018) and their NPS equivalents?

The experience over the last decade tells us that when it comes to new psychoactive substances, we should expect the unusual and unexpected. While it is easy to speculate about what might happen, detecting what actually happens next depends on strong early warning systems. (Undoubtedly, our understanding of the epidemiology of NPS use also needs to improve.)

Early warning systems can play a central role in situational awareness, preparedness, and responses to health and social threats caused by NPS. Yet, like all public health interventions, strengthening these systems is a continuous process, and work remains to be done. The recent developments in the NPS market serves to highlight the importance of continued investment in strong early warning systems at both national and EU level, as well as a more rapid risk assessment process at EU level, in order to help protect the health and security of people living in Europe. The new legislative framework that will apply from November 2018 offers the European Union a major tool in helping achieve these aims.

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References

96/750/JHA (1996) Joint Action of 17 December 1996 adopted by the Council on the basis of Article K.3 of the Treaty on European Union concerning the approximation of the laws and practices of the Member States of the European Union to combat drug addiction and to prevent and combat illegal drug trafficking. Off J Eur Union L342:6–8. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:31996F0750

97/396/JHA (1997) Joint Action of 16 June 1997 adopted by the Council on the basis of Article K.3 of the Treaty on European Union, concerning the information exchange, risk assessment and the control of new synthetic drugs. Off J Eur Union L167:1–3. https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:31997F0396

Abbate V, Kicman AT, Evans-Brown M, McVeigh J, Cowan DA, Wilson C, Coles SJ, Walker CJ (2015) Anabolic steroids detected in bodybuilding dietary supplements – a significant risk to public health. Drug Test Anal 7:609–618. https://doi.org/10.1002/dta.1728

Adamowicz P (2016) Fatal intoxication with synthetic cannabinoid MDMB-CHMICA. Forensic Sci Int 261:e5–e10. https://doi.org/10.1016/j.forsciint.2016.02.024

Anonymous (1953) Esters of morphine. Bull Narc 4:36–38 https://www.unodc.org/unodc/en/data-and-analysis/bulletin/bulletin_1953-01-01_2_page009.html

Auwärter V, Dresen S, Weinmann W, Muller M, Putz M, Ferreiros N (2009) 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? J Mass Spectrom 44:832–837. https://doi.org/10.1002/jms.1558

Baird CR, Fox P, Colvin LA (2014) Gabapentinoid abuse in order to potentiate the effect of methadone: a survey among substance misusers. Eur Addict Res 20:115–118. https://doi.org/10.1159/000355268

Bakota EL, Samms WC, Gray TR, Oleske DA, Hines MO (2018) Case reports of fatalities involving tianeptine in the United States. J Anal Toxicol. https://doi.org/10.1093/jat/bky023

Banister SD, Longworth M, Kevin R, Sachdev S, Santiago M, Stuart J, Mack JB, Glass M, McGregor IS, Connor M, Kassiou M (2016) Pharmacology of valinate and tert-leucinate synthetic cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and their analogues. ACS Chem Neurosci 7:1241–1254. https://doi.org/10.1021/acschemneuro.6b00137

Baum RM (1985) New variety of street drugs poses growing problem. Chem Eng News 63:7–16

Beck J, Rosenbaum M (1994) Pursuit of ecstasy: the MDMA experience. State University of New York Press, Albany

Berens AI, Voets AJ, Demedts P (1996) Illicit fentanyl in Europe. Lancet 347:1334–1335. https://doi.org/10.1016/S0140-6736(96)90981-2

Berridge V (2013) Demons: our changing attitudes to alcohol, tobacco, and drugs. Oxford University Press, Oxford

Berridge V, Edwards G (1981) Opium and the people: opiate use in nineteenth-century England. Allen Lane, Penguin Books Ltd., London

Blackman S, Bradley R (2017) From niche to stigma-Headshops to prison: exploring the rise and fall of synthetic cannabinoid use among young adults. Int J Drug Policy 40:70–77. https://doi.org/10.1016/j.drugpo.2016.10.015

Brandt S, King L, Evans-Brown M (2014) The new drug phenomenon. Drug Test Anal 6:587–597. https://doi.org/10.1002/dta.1686

Brunn K (1975) Gentleman's Club: international control of drugs and alcohol. University of Chicago Press, Chicago

Cohen PA, Venhuis BJ, Brandt SD (2016) Advancing supplement science: challenges and solutions. Drug Test Anal 8(3–4):e1–e3. https://doi.org/10.1002/dta.1953

Collin M (1997) The story of ecstasy culture and acid house. Serpent's Tail, London

Convention on Psychotropic Substances (1971) http://www.unodc.org/unodc/en/treaties/psychotropics.html. Accessed 11 Jun 2018

Cooper DA (1988) Future synthetic drugs of abuse. In: Castonguay RT (ed) In: Proceedings of the international symposium on the forensic aspects of controlled substances: March 28–April 1. Laboratory Division, Federal Bureau of Investigation, US Dept. of Justice, Washington, DC, pp 79–103

Cooper ZD (2016) Adverse effects of synthetic cannabinoids: management of acute toxicity and withdrawal. Curr Psychiatry Rep 18:52. https://doi.org/10.1007/s11920-11016-10694-11921

Council Decision 2005/387/JHA of 10 May 2005 on the information exchange, risk-assessment and control of new psychoactive substances (2005) Off J Eur Union L127:32–37. https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32005D0387

Courtwright DT (2002) Forces of habit drugs and the making of the modern world. Harvard University Press, Cambridge

Cox BM (2011) Pharmacology of opioid drugs. In: Pasternak G (ed) The opiate receptors. Springer, New York, pp 23–58

Dahan A, Sarton E, Teppema L, Olievier C, Nieuwenhuijs D, Matthes HW, Kieffer BL (2001) Anesthetic potency and influence of morphine and sevoflurane on respiration in mu-opioid receptor knockout mice. Anesthesiology 94:824–832 http://anesthesiology.pubs.asahq.org/article.aspx?articleid=1944782

Dahan A, Aarts L, Smith TW (2010) Incidence, reversal, and prevention of opioid-induced respiratory depression. Anesthesiology 112:226–238. https://doi.org/10.1097/ALN.0b013e3181c38c25

de Boer D, Goemans WP, Ghezavat VR, van Ooijen RD, Maes RA (2003) Seizure of illicitly produced Para-fluorofentanyl: quantitative analysis of the content of capsules and tablets. J Pharm Biomed Anal 31:557–562. https://doi.org/10.1016/S0731-7085(02)00684-2

Decision No 1082/2013/EU of the European Parliament and of the Council of 22 October 2013 on serious cross-border threats to health and repealing Decision No 2119/98/EC (2013) Off J Eur Union L293:1–15. https://eur-lex.europa.eu/legal-content/GA/ALL/?uri=celex:32013D1082

Dekker S (2014) Safety differently: human factors for a new era, 2nd edn. CRC Press, Boca Raton

Dempsey SK, Poklis JL, Sweat K, Cumpston K, Wolf CE (2017) Acute toxicity from intravenous use of the tricyclic antidepressant tianeptine. J Anal Toxicol 41:547–550. https://doi.org/10.1093/jat/bkx034

Deprez EE, Hui L, Wills K (2018) Deadly Chinese fentanyl is creating a new era of drug kingpins. Bloomberg. https://www.bloomberg.com/news/features/2018-05-22/deadly-chinese-fentanyl-is-creating-a-new-era-of-drug-kingpins. Accessed 11 June 2018

Dion M, AbdelMalik P, Mawudeku A (2015) Big data and the global public health intelligence network (GPHIN). Can Commun Dis Rep 41:209–214

Directive (EU) 2017/2103 of the European Parliament and of the Council of 15 November 2017 amending Council Framework Decision 2004/757/JHA in order to include new psychoactive substances in the definition of 'drug' and repealing Council Decision 2005/387/JHA (2017) Off J Eur Union L305:12–18. https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:32017L2103

Directive 2011/62/EU of the European Parliament and of the Council of 8 June 2011 amending Directive 2001/83/EC on the Community code relating to medicinal products for human use, as regards the prevention of the entry into the legal supply chain of falsified medicinal products

(2011) Off J Eur Union L174:74–87. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32011L0062

Dobaja M, Grenc D, Kozelj G, Brvar M (2017) Occupational transdermal poisoning with synthetic cannabinoid cumyl-PINACA. Clin Toxicol (Phila) 55:193–195. https://doi.org/10.1080/15563650.2016.1278224

Dyck L (2008) Psychedelic psychiatry. LSD from clinic to campus. John Hopkins University Press, Baltimore

Elliott SP, Hernandez Lopez E (2018) A series of deaths involving carfentanil in the UK and associated post-mortem blood concentrations. J Anal Toxicol. https://doi.org/10.1093/jat/bkx109

Ernst L, Brandhorst K, Papke U, Altrogge A, Zodel S, Langer N, Beuerle T (2017) Identification and quantification of synthetic cannabinoids in 'spice-like' herbal mixtures: update of the German situation in early 2017. Forensic Sci Int 277:51–58. https://doi.org/10.1016/j.forsciint.2017.05.019

Estievenart G (1995) Policies and strategies to combat drugs in Europe. The treaty on European Union: framework for a new European strategy to combat drugs? Martinus Nijhoff, Dordrecht

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2007) Early-warning system on new psychoactive substances. Operating guidelines. Publications Office of the European Union, Luxembourg. https://publications.europa.eu/en/publication-detail/-/publication/08497900-eaab-45fb-b918-b1697f90e8b8/language-en. Accessed 11 June 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2009) Understanding the 'Spice' phenomenon. Office for Official Publications of the European Communities, Luxembourg. doi: https://doi.org/10.2810/27063. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2010) Risk assessment of new psychoactive substances. Operating guidelines. Publications Office of the European Union, Luxembourg. doi:https://doi.org/10.2810/2428. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2014a) Report on the risk assessment of 4-methylamphetamine in the framework of the council decision on new psychoactive substances, Publications Office of the European Union, Luxembourg. doi: https://doi.org/10.2810/86512. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2014b) Report on the risk assessment of 3,4-dichloro-N-{[1-(dimethylamino)cyclohexyl]methyl}benzamide (AH-7921) in the framework of the council decision on new psychoactive substances. Publications Office of the European Union, Luxembourg. doi: https://doi.org/10.2810/5691. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2016a) EU drug markets report. In: Depth analysis. Luxembourg, Publications Office of the European Union. doi: https://doi.org/10.2810/219411. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2016b) EMCDDA–Europol joint report on a new psychoactive substance: N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl] acetamide (acetylfentanyl). Luxembourg, Publications Office of the European Union. doi: https://doi.org/10.2810/890694. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2016c) Preventing opioid overdose deaths with take-home naloxone. Luxembourg, Publications Office of the European Union. doi: https://doi.org/10.2810/357062. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2017a) Report on the risk assessment of N-(1-phenethylpiperidin-4-yl)-N-phenylacrylamide (Acryloylfentanyl) in the framework of the council decision on new psychoactive substances. Publications Office of the European Union, Luxembourg. doi: https://doi.org/10.2810/16112. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2017b) Synthetic cannabinoids in Europe. http://www.emcdda.europa.eu/topics/pods/synthetic-cannabinoids_en. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2017c) Report on the risk assessment of methyl 2-[[1-(cyclohexylmethyl)-1H-indole-3- carbonyl]amino]-3,3-

dimethylbutanoate (MDMB-CHMICA) in the framework of the council decision on new psychoactive substances. Publications Office of the European Union, Luxembourg. doi: https://doi.org/10.2810/08132. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2017d) EMCDDA–Europol joint report on a new psychoactive substance: methyl 1-(2-phenylethyl)-4-phenyl (propanoyl)aminopiperidine-4-carboxylate (carfentanil): in accordance with article 5 of council decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. Publications Office of the European Union, Luxembourg. doi: https://doi.org/10.2810/282222. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2018a) European drug report: trends and developments. Publications Office of the European Union, Luxembourg. doi: https://doi.org/10.2810/800331. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2018b) Fentanils and synthetic cannabinoids: driving greater complexity into the drug situation. An update from the EU early warning system (June 2018). Publications Office of the European Union, Luxembourg. doi: https://doi.org/10.2810/603753. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2018c) Mission. http://www.emcdda.europa.eu/about/mission. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2018d) Reitox network. http://www.emcdda.europa.eu/about/partners/reitox-network. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2018e) Action on new drugs. http://www.emcdda.europa.eu/activities/action-on-new-drugs. Accessed 11 Jun 2018

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and Eurojust (2016) New psychoactive substances in Europe: legislation and prosecution – current challenges and solutions. Publications Office of the European Union, Luxembourg. doi: https://doi.org/10.2810/777512. Accessed 11 Jun 2018

Evans-Brown M, Sedefov R (2017) New psychoactive substances: driving greater complexity into the drug problem. Addiction 112:36–38. https://doi.org/10.1111/add.13528

Evans-Brown M, Dawson RT, Chandler M, McVeigh J (2009) Use of melanotan I and II in the general population. BMJ 338:b566. https://doi.org/10.1136/bmj.b566

Evans-Brown M, Kimergård A, McVeigh J, Chandler M, Brandt SD (2014) Is the breast cancer drug tamoxifen being sold as a bodybuilding dietary supplement? Br Med J 348:g1476. https://doi.org/10.1136/bmj.g1476

Faul M, Lurie P, Kinsman JM, Dailey MW, Crabaugh C, Sasser SM (2017) Multiple naloxone administrations among emergency medical service providers is increasing. Prehosp Emerg Care 21:411–419. https://doi.org/10.1080/10903127.2017.1315203

Ford LT, Berg JD (2018) Analytical evidence to show letters impregnated with novel psychoactive substances are a means of getting drugs to inmates within the UK prison service. Ann Clin Biochem. https://doi.org/10.1177/0004563218767462

Ford BM, Tai S, Fantegrossi WE, Prather PL (2017) Synthetic pot: not your grandfather's marijuana. Trends Pharmacol Sci 38:257–276. https://doi.org/10.1016/j.tips.2016.12.003

Frinculescu A, Lyall CL, Ramsey J, Miserez B (2017) Variation in commercial smoking mixtures containing third-generation synthetic cannabinoids. Drug Test Anal 9:327–333. https://doi.org/10.1002/dta.1975

Fritschi G, Klein B (1995) Zwischen- und nebenproduckte bei der illegalen herstellung von fentanyl und fluorofentanylen und die synthese hirer acetylhomologen. Arch Kriminol 196:149–155

Gaoni Y, Mechoulam R (1964) Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc 86:1646–1647. https://doi.org/10.1021/ja01062a046

Gladden RM, Martinez P, Seth P (2016) Fentanyl law enforcement submissions and increases in synthetic opioid-involved overdose deaths – 27 states, 2013–2014. MMWR Morb Mortal Wkly Rep 65:837–843. https://doi.org/10.15585/mmwr.mm6533a2

Griffiths P, Evans-Brown M, Sedefov R (2013) Getting up to speed with the public health and regulatory challenges posed by new psychoactive substances in the information age. Addiction 108:1700–1703. https://doi.org/10.1111/add.12287

Guerrieri D, Rapp E, Roman M, Druid H, Kronstrand R (2017a) Postmortem and toxicological findings in a series of furanylfentanyl-related deaths. J Anal Toxicol 41:242–249. https://doi.org/10.1093/jat/bkw129

Guerrieri D, Rapp E, Roman M, Thelander G, Kronstrand R (2017b) Acrylfentanyl: another new psychoactive drug with fatal consequences. Forensic Sci Int 277:e21–e29. https://doi.org/10.1016/j.forsciint.2017.05.010

Häkkinen M, Vuori E, Kalso E, Gergov M, Ojanpera I (2014) Profiles of pregabalin and gabapentin abuse by postmortem toxicology. Forensic Sci Int 241:1–6. https://doi.org/10.1016/j.forsciint.2014.04.028

Halford B (2015) A glimpse inside the sophisticated world of synthetic cannabinoids. Chem Eng News. http://cen.acs.org/articles/93/web/2015/04/Glimpse-Inside-Sophisticated-World-Synthetic.html. Accessed 11 June 2018

Hartley DM, Nelson NP, Arthur RR, Barboza P, Collier N, Lightfoot N, Linge JP, van der Goot E, Mawudeku A, Madoff LC, Vaillant L, Walters R, Yangarber R, Mantero J, Corley CD, Brownstein JS (2013) An overview of internet biosurveillance. Clin Microbiol Infect 19:1006–1013. https://doi.org/10.1111/1469-0691.12273

Helander A, Backberg M, Signell P, Beck O (2017) Intoxications involving acrylfentanyl and other novel designer fentanyls – results from the Swedish STRIDA project. Clin Toxicol (Phila) 55:589–599. https://doi.org/10.1080/15563650.2017.1303141

Henderson GL (1988) Designer drugs: past history and future prospects. J Forensic Sci 33:569–575

Henderson GL (1991) Fentanyl-related deaths: demographics, circumstances, and toxicology of 112 cases. J Forensic Sci 36:422–433

Her Majesty's Inspectorate of Prisons (HMIP) (2015) Changing patterns of substance misuse in adult prisons and service responses. Her Majesty's Inspectorate of Prisons, London. https://www.justiceinspectorates.gov.uk/hmiprisons/wp-content/uploads/sites/4/2015/12/Substance-misuse-web-2015.pdf. Accessed 11 Jun 2018

Herzberg D (2010) Happy pills in America: from Miltown to Prozac. Johns Hopkins University Press, Baltimore

Herzberg D (2012) Busted for blockbusters: "scrip mills", quaaludes, and prescribing power in the 1970s. In: Greene J, Watkins E (eds) Prescribed: writing, filling, using, and abusing the prescription in modern America. Johns Hopkins University Press, Baltimore, pp 207–231

Hikin L, Smith PR, Ringland E, Hudson S, Morley SR (2018) Multiple fatalities in the North of England associated with synthetic fentanyl analogue exposure: detection and quantitation a case series from early 2017. Forensic Sci Int 282:179–183. https://doi.org/10.1016/j.forsciint.2017.11.036

Hill SL, Najafi J, Dunn M, Acheampong P, Kamour A, Grundlingh J, Blain PG, Thomas SH (2016) Clinical toxicity following analytically confirmed use of the synthetic cannabinoid receptor agonist MDMB-CHMICA. A report from the identification of novel psychoActive substances (IONA) study. Clin Toxicol (Phila) 54:638–643. https://doi.org/10.1080/15563650.2016.1190980

Horth RZ, Crouch B, Horowitz BZ, Prebish A, Slawson M, McNair J, Elsholz C, Gilley S, Robertson J, Risk I, Hill M, Fletcher L, Hou W, Peterson D, Adams K, Vitek D, Nakashima A, Dunn A (2018) Acute poisonings from a synthetic cannabinoid sold as cannabidiol – Utah, 2017–2018. MMWR Morb Mortal Wkly Rep 67:587–588. https://doi.org/10.15585/mmwr.mm6720a5

Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET, Frank RA (2001) Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. Arch Gen Psychiatry 58:322–328. https://doi.org/10.1001/archpsyc.58.4.322

Jack H (2009) The story of spice. Financial times. https://www.ft.com/content/1721e2da-f8a0-11dd-aae8-000077b07658. Accessed 11 Jun 2018

Janssen PA (1982) Potent, new analgesics, tailor-made for different purposes. Acta Anaesthesiol Scand 26:262–268. https://doi.org/10.1111/j.1399-6576.1982.tb01765.x

Janssen PAJ, Van der Eycken CAM (1968) The chemical anatomy of potent morphine-like analgesics. In: Burger A (ed) Drugs affecting the central nervous system, vol 2. Marcel Dekker, New York, pp 25–60

Kasper AM, Ridpath AD, Arnold JK, Chatham-Stephens K, Morrison M, Olayinka O, Parker C, Galli R, Cox R, Preacely N, Anderson J, Kyle PB, Gerona R, Martin C, Schier J, Wolkin A, Dobbs T (2015) Severe illness associated with reported use of synthetic cannabinoids – Mississippi, April 2015. MMWR Morb Mortal Wkly Rep 64:1121–1122. https://doi.org/10.15585/mmwr.mm6439a7

Kieffer BL (1999) Opioids: first lessons from knockout mice. Trends Pharmacol Sci 20:19–26. https://doi.org/10.1016/S0165-6147(98)01279-6

Kim HK, Nelson LS (2015) Reducing the harm of opioid overdose with the safe use of naloxone: a pharmacologic review. Expert Opin Drug Saf 14:1137–1146. https://doi.org/10.1517/14740338.2015.1037274

Klar SA, Brodkin E, Gibson E, Padhi S, Predy C, Green C, Lee V (2016) Furanyl-fentanyl overdose events caused by smoking contaminated crack cocaine – British Columbia, Canada, July 15–18, 2016. MMWR Morb Mortal Wkly Rep 65:1015–1016. https://doi.org/10.15585/mmwr.mm6537a6

Kram TC, Cooper DA, Allen AC (1981) Behind the identification of China white. Anal Chem 53:1379A–1386A

Kronstrand R, Druid H, Holmgren P, Rajs J (1997) A cluster of fentanyl-related deaths among drug addicts in Sweden. Forensic Sci Int 88:185–195. https://doi.org/10.1016/S0379-0738(97)00068-6

Langer N, Lindigkeit R, Schiebel HM, Ernst L, Beuerle T (2014) Identification and quantification of synthetic cannabinoids in 'spice-like' herbal mixtures: a snapshot of the German situation in the autumn of 2012. Drug Test Anal 6:59–71. https://doi.org/10.1002/dta.1499

Langer N, Lindigkeit R, Schiebel HM, Papke U, Ernst L, Beuerle T (2016) Identification and quantification of synthetic cannabinoids in "spice-like" herbal mixtures: update of the German situation for the spring of 2016. Forensic Sci Int 269:31–41. https://doi.org/10.1016/j.forsciint.2016.10.023

Levinson M (2016) The box: how the shipping container made the world smaller and the world economy bigger. In: Princeton University Press, Princeton

Linge JP, Mantero J, Fuart F, Belyaeva J, Atkinson M, van der Goot E (2012) Tracking media reports on the Shiga toxin-producing Escherichia coli O104: H4 outbreak in Germany. In: Kostkova P, Szomszor M, Fowler D (eds) Electronic healthcare. Springer, Berlin, pp 178–185. https://doi.org/10.1007/978-3-642-29262-0_26

Macfarlane V, Christie G (2015) Synthetic cannabinoid withdrawal: a new demand on detoxification services. Drug Alcohol Rev 34:147–153. https://doi.org/10.1111/dar.12225

Macleod DB, Habib AS, Ikeda K, Spyker DA, Cassella JV, Ho KY, Gan TJ (2012) Inhaled fentanyl aerosol in healthy volunteers: pharmacokinetics and pharmacodynamics. Anesth Analg 115:1071–1077. https://doi.org/10.1213/ANE.0b013e3182691898

Mantero J, Szegedi E, Payne Hallstrom L, Lenglet A, Depoortere E, Kaic B, Blumberg L, Linge JP, Coulombier D (2014) Enhanced epidemic intelligence using a web-based screening system during the 2010 FIFA World Cup in South Africa. Euro Surveill 19(18):20796 http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20796

Marraffa JM, Stork CM, Hoffman RS, Su MK (2018) Poison control center experience with tianeptine: an unregulated pharmaceutical product with potential for abuse. Clin Toxicol (Phila):1–4. https://doi.org/10.1080/15563650.2018.1476694

Martin J, Cunliffe J, Décary-Hétu D, Aldridge J (2018) Effect of restricting the legal supply of prescription opioids on buying through online illicit marketplaces: interrupted time series analysis. BMJ 361:k2270. https://doi.org/10.1136/bmj.k2270

McAllister WB (1999) Drug diplomacy in the twentieth century: an international history. Routledge, Abingdon
McNamara S, Stokes S, Kilduff R, Shine A (2015) Pregabalin abuse amongst opioid substitution treatment patients. Ir Med J 108:309–310
Meyers FH, Rose AJ, Smith DE (1968) Incidents involving the Haight-Ashbury population and some uncommonly used drugs. J Psychedelic Drugs 1:139–146
Miller RJ (2014) Drugged: the science and culture behind psychotropic drugs. University Press, Oxford
Moosmann B, Angerer V, Auwärter V (2015) Inhomogeneities in herbal mixtures: a serious risk for consumers. Forensic Toxicol 33:54–60. https://doi.org/10.1007/s11419-014-0247-4
Moritz E, Austin C, Wahl M, DesLauriers C, Navon L, Walblay K, Hendrickson M, Phillips A, Kerins J, Pennington AF, Lavery AM, El Zahran T, Kauerauf J, Yip L, Thomas J, Layden J (2018) Notes from the field: outbreak of severe illness linked to the vitamin K antagonist brodifacoum and use of synthetic cannabinoids – Illinois, March–April 2018. MMWR Morb Mortal Wkly Rep 67:607–608. https://doi.org/10.15585/mmwr.mm6721a4
Morris H (2012) Criminal chlorination. An interview with a clandestine chemist. Vice. http://www.vice.com/read/criminal-chlorination-0000350-v19n9. Accessed 11 Jun 2018
Morris H, Wallach J (2014) From PCP to MXE: a comprehensive review of the non-medical use of dissociative drugs. Drug Test Anal 6:614–632
Musto DF (1973) American disease: origins of narcotic control. Yale University Press, New York
National Crime Agency (2018) Organised crime group mixed potentially lethal drug fentanyl and posted it around the world. http://www.nationalcrimeagency.gov.uk/index.php/news-media/nca-news/1367-organised-crime-group-mixed-potentially-lethal-drug-fentanyl-and-posted-it-around-the-world. Accessed 11 Jun 2018
New York Times (1968) Synthetic marijuana being banned by U.S. New York Times, New York
O'Donnell JK, Gladden RM, Seth P (2017) Trends in deaths involving heroin and synthetic opioids excluding methadone, and law enforcement drug product reports, by census region – United States, 2006–2015. MMWR Morb Mortal Wkly Rep 66:897–903. https://doi.org/10.15585/mmwr.mm6634a2
Ojanperä I, Gergov M, Rasanen I, Lunetta P, Toivonen S, Tiainen E, Vuori E (2006) Blood levels of 3-methylfentanyl in 3 fatal poisoning cases. Am J Forensic Med Pathol 27:328–331. https://doi.org/10.1097/01.paf.0000188097.78132.e5
Ojanperä I, Gergov M, Liiv M, Riikoja A, Vuori E (2008) An epidemic of fatal 3-methylfentanyl poisoning in Estonia. Int J Legal Med 122:395–400. https://doi.org/10.1007/s00414-008-0230-x
Olcott A (2012) Open source intelligence in a networked world. Continuum, London
Owen DR, Wood DM, Archer JR, Dargan PI (2016) Phenibut (4-amino-3-phenyl-butyric acid): availability, prevalence of use, desired effects and acute toxicity. Drug Alcohol Rev 35:591–596. https://doi.org/10.1111/dar.12356
Pap C (2016) ADB-Fubinaca in the real world: a case series of 15 poisonings. 36th international congress of the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) 24–27 May 2016, Madrid, Spain. Clin Toxicol (Phila) 54:384. https://doi.org/10.3109/15563650.2016.1165952
Pap C (2018) 5F-MDMB-PINACA on the streets: case series of 17 poisonings. 38th international congress of the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) 22–25 May 2018, Bucharest, Romania. Clin Toxicol (Phila) 56:51. https://doi.org/10.1080/15563650.2018.1457818
Passie T, Benzenhofer U (2016) The history of MDMA as an underground drug in the United States, 1960–1979. J Psychoactive Drugs 48:67–75
Passie T, Benzenhofer U (2018) MDA, MDMA, and other "mescaline-like" substances in the US military's search for a truth drug (1940s to 1960s). Drug Test Anal 10:72–80
Pasternak GW, Pan YX (2013) Mu opioids and their receptors: evolution of a concept. Pharmacol Rev 65:1257–1317. https://doi.org/10.1124/pr.112.007138

Pattinson KT (2008) Opioids and the control of respiration. Br J Anaesth 100:747–758. https://doi.org/10.1093/bja/aen094

Peace MR, Krakowiak RI, Wolf CE, Poklis A, Poklis JL (2017) Identification of MDMB-FUBINACA in commercially available e-liquid formulations sold for use in electronic cigarettes. Forensic Sci Int 271:92–97. https://doi.org/10.1016/j.forsciint.2016.12.031

Pertwee RG (2005) The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. AAPS J 7:E625–E654. https://doi.org/10.1208/aapsj070364

Pertwee RG (2015) Endocannabinoids. In: Handbook of experimental pharmacology. Springer, Cham

Pertwee RG, Cascio MG (2014) Known pharmacological actions of delta-9-tetrahydrocannabinol and four other chemical constituents of cannabis that activate cannabinoid receptors. In: Pertwee R (ed) Handbook of Cannabis. Oxford University Press, Oxford, pp 115–136

Poortman-van der Meer AJ, Huizer H (1996) First encounter with p-fluorofentanyl in the Netherlands. Toxichem Krimtech 63:7–14

Proposal for a Council Decision on the information exchange, risk-assessment and the control on new narcotic drugs and new synthetic drugs 2003/0215 (CNS) (2003) http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52003PC0560. Accessed 11 Jun 2018

Quintana P, Ventura M, Grifell M, Palma A, Galindo L, Fornís I, Gil C, Carbón X, Caudevilla F, Farré M, Torrens M (2017) The hidden web and the fentanyl problem: detection of ocfentanil as an adulterant in heroin. Int J Drug Policy 40:78–83. https://doi.org/10.1016/j.drugpo.2016.10.006

Ralphs R, Williams L, Askew R, Norton A (2017) Adding spice to the porridge: the development of a synthetic cannabinoid market in an English prison. Int J Drug Policy 40:57–69. https://doi.org/10.1016/j.drugpo.2016.10.003

Rasmussen N (2008a) America's first amphetamine epidemic 1929-1971: a quantitative and qualitative retrospective with implications for the present. Am J Public Health 98:974–985. https://doi.org/10.2105/AJPH.2007.110593

Rasmussen N (2008b) On speed. From Benzedrine to Adderall. NYU Press, New York

Rasmussen N (2012) Goofball panic. Barbiturates, “dangerous” and addictive drugs, and the regulation of medicine in postwar America. In: Greene J, Watkins E (eds) Prescribed: writing, filling, using, and abusing the prescription in modern America. Johns Hopkins University Press, Baltimore, pp 23–45

Reggio PH (ed) (2009) The cannabinoids receptors. Humana Press, New York

Regulation (EC) No 1920/2006 of the European Parliament and of the Council of 12 December 2006 on the European Monitoring Centre for Drugs and Drug Addiction (recast) (2006) Off J Eur Union L376:1–13. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32006R1920

Regulation (EU) 2017/2101 of the European Parliament and of the Council of 15 November 2017 amending Regulation (EC) No 1920/2006 as regards information exchange on, and an early warning system and risk assessment procedure for, new psychoactive substances (2017) Off J Eur Union L305:1–7. https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32017R2101

Reynolds S (1999) Generation ecstasy: into the world of techno and rave culture. Routledge, New York

Richards WA (2016) Secret knowledge. Psychedelics and religious experiences. Columbia University Press, New York

Romberg R, Sarton E, Teppema L, Matthes HW, Kieffer BL, Dahan A (2003) Comparison of morphine-6-glucuronide and morphine on respiratory depressant and antinociceptive responses in wild type and mu-opioid receptor deficient mice. Br J Anaesth 91:862–870. https://doi.org/10.1093/bja/aeg279

Samuels BA, Nautiyal KM, Kruegel AC, Levinstein MR, Magalong VM, Gassaway MM, Grinnell SG, Han J, Ansonoff MA, Pintar JE, Javitch JA, Sames D, Hen R (2017) The behavioral effects of the antidepressant tianeptine require the mu-opioid receptor. Neuropsychopharmacology 42:2052–2063. https://doi.org/10.1038/npp.2017.60

San Francisco Department of Public Health (SFDPH) (2015) Severe opioid overdoses in San Francisco caused by fentanyl-containing "Xanax" pill. http://www.sfcdcp.org/document.html?id=1005. Accessed 11 Jun 2018

Savulescu J, Meulen RT, Kahane G (eds) (2011) Enhancing human capacities. Wiley, Chichester

Schäffer M, Gröger T, Pütz M, Zimmermann R (2013) Assessment of the presence of damiana in herbal blends of forensic interest based on comprehensive two-dimensional gas chromatography. Forensic Toxicol 31:251–262. https://doi.org/10.1007/s11419-013-0186-5

Schäper J (2016) Wirkstoffgehalte und inhomogene Verteilung des Wirkstoffs MDMB-CHMICA in Kräutermischungen. Toxichem Krimtech 83:112–114

Schivelbusch W (1993) Tastes of paradise. A social history of spices, stimulants, and intoxicants. Vintage Books, New York

Schumann H, Erickson T, Thompson TM, Zautcke JL, Denton JS (2008) Fentanyl epidemic in Chicago, Illinois and surrounding Cook County. Clin Toxicol (Phila) 46:501–506. https://doi.org/10.1080/15563650701877374

Shevyrin V, Melkozerov V, Nevero A, Eltsov O, Shafran Y, Morzherin Y, Lebedev AT (2015) Identification and analytical characteristics of synthetic cannabinoids with an indazole-3-carboxamide structure bearing a N-1-methoxycarbonylalkyl group. Anal Bioanal Chem 407:6301–6315. https://doi.org/10.1007/s00216-015-8612-7

Shulgin AT (1975) Drugs of abuse in the future. Clin Tox (Phila) 8:405–456

Single Convention on Narcotic Drugs 1961 as amended by the 1972 Protocol (1961) https://www.unodc.org/unodc/en/treaties/single-convention.html. Accessed 11 Jun 2018

Smil V (2010) Prime movers of globalization. The history and impact of diesel engines and gas turbines. MIT Press, Cambridge

Sneader W (2005) Drug discovery: a history. Wiley, Chichester. https://doi.org/10.1002/0470015535

Södertörns Tingsrätt (2018) Två bröder döms för åtta fall av grovt vållande till annans död för försäljning av fentanylanaloger på internet. http://www.sodertornstingsratt.domstol.se/Om-tingsratten/Nyheter-och-pressmeddelanden/Tva-broder-doms-for-atta-fall-av-grovt-vallande-till-annans-dod-for-forsaljning-av-fentanylanaloger-pa-internet/. Accessed 11 Jun 2018

Somerville NJ, O'Donnell J, Gladden RM, Zibbell JE, Green TC, Younkin M, Ruiz S, Babakhanlou-Chase H, Chan M, Callis BP, Kuramoto-Crawford J, Nields HM, Walley AY (2017) Characteristics of fentanyl overdose – Massachusetts, 2014–2016. MMWR Morb Mortal Wkly Rep 66:382–386. https://doi.org/10.15585/mmwr.mm6614a2

Sorokin VI (1994) Clandestine synthesis of 3-methylfentanyl. J Clan Lab Invest Chem Assoc 4:28–29

Sorokin VI, Semkin EP, Savilov AP (1994) Expert examination of 3-methylfentanyl. Microgram 27:221–226

Stearns EL (2011) Electronic value exchange. Origins of the VISA electronic payment system. Springer, London. https://doi.org/10.1007/978-1-84996-139-4

Sumnall H (2016) Epidemiology of use of novel psychoactive substances. In: von Heyden M, Jungaberle H, Majić T (eds) Handbuch psychoaktive substanzen. Springer, Berlin. https://doi.org/10.1007/978-3-642-55214-4_11-1

Sumnall HR, Evans-Brown M, McVeigh J (2011) Social, policy, and public health perspectives on new psychoactive substances. Drug Test Anal 3:515–523. https://doi.org/10.1002/dta.310

Sutter ME, Gerona RR, Davis MT, Roche BM, Colby DK, Chenoweth JA, Adams AJ, Owen KP, Ford JB, Black HB, Albertson TE (2017) Fatal fentanyl: one pill can kill. Acad Emerg Med 24:106–113. https://doi.org/10.1111/acem.13034

Tai S, Fantegrossi WE (2017) Pharmacological and toxicological effects of synthetic cannabinoids and their metabolites. In: Baumann MH, Glennon RA, Wiley JL (eds) Neuropharmacology of New Psychoactive Substances (NPS): the science behind the headlines. Springer, Cham, pp 249–262. https://doi.org/10.1007/7854_2016_60

Tait RJ, Caldicott D, Mountain D, Hill SL, Lenton S (2016) A systematic review of adverse events arising from the use of synthetic cannabinoids and their associated treatment. Clin Toxicol (Phila) 54:1–13. https://doi.org/10.3109/15563650.2015.1110590

Tapp L, Ramsey JG, Wen A, Gerona R (2017) Synthetic cannabinoid and mitragynine exposure of law enforcement agents during the raid of an illegal laboratory – Nevada, 2014. MMWR Morb Mortal Wkly Rep 66:1291–1294. https://doi.org/10.15585/mmwr.mm6647a3

Tomassoni AJ, Hawk KF, Jubanyik K, Nogee DP, Durant T, Lynch KL, Patel R, Dinh D, Ulrich A, D'Onofrio G (2017) Multiple fentanyl overdoses – new haven, Connecticut, June 23, 2016. MMWR Morb Mortal Wkly Rep 66(4):107–111. https://doi.org/10.15585/mm6604a4

Tone A (2008) The age of anxiety: a history of America's turbulent affair with tranquilizers. Basic Books, New York

Trecki J, Gerona RR, Schwartz MD (2015) Synthetic cannabinoid-related illnesses and deaths. N Engl J Med 373:103–107. https://doi.org/10.1056/NEJMp1505328

Uddayasankar U, Lee C, Oleschuk C, Eschun G, Ariano RE (2018) The pharmacokinetics and pharmacodynamics of carfentanil after recreational exposure: a case report. Pharmacotherapy 38 (6):e41–e45. https://doi.org/10.1002/phar.2117

Ujváry I, Jorge R, Christie R, Le Ruez T, Danielsson HV, Kronstrand R, Elliott S, Gallegos A, Sedefov R, Evans-Brown M (2017) Acryloylfentanyl, a recently emerged new psychoactive substance: a comprehensive review. Forensic Toxicol 35:232–243. https://doi.org/10.1007/s11419-017-0367-8

United States Centers for Disease Control and Prevention (US CDC) (2015) Increases in fentanyl drug confiscations and fentanyl-related overdose fatalities. CDCHAN-00384. https://emergency.cdc.gov/han/han00384.asp. Accessed 11 Jun 2018

United States Centers for Disease Control and Prevention (US CDC) (2018) Outbreak of life-threatening coagulopathy associated with synthetic cannabinoids use. CDCHAN-00410. https://emergency.cdc.gov/han/han00410.asp. Accessed 11 Jun 2018

United States Department of Justice (US DOJ) (2018) Schedules of controlled substances: temporary placement of fentanyl-related substances in schedule I. Fed Regist 83:5188–5192. https://www.federalregister.gov/d/2018-02319. Accessed 11 Jun 2018

United States InterAgency Board for Equipment Standardization and Interoperability (US IABESI) (2017) Recommendations on selection and use of personal protective equipment and decontamination products for first responders against exposure hazards to synthetic opioids, including fentanyl and fentanyl analogues. https://www.interagencyboard.org/sites/default/files/publications/IAB First Responder PPE and Decontamination Recommendations for Fentanyl.pdf. Accessed 11 Jun 2018

United States White House National Security Council (US WHNSC) (2017) Fentanyl safety recommendations for first responders. https://www.whitehouse.gov/sites/whitehouse.gov/files/images/Final%20STANDARD%20size%20of%20Fentanyl%20Safety%20Recommendations%20for%20First%20Respond. . ..pdf. Accessed 11 Jun 2018

User Voice (2016) Spice: the bird killer – what prisoners think about the use of spice and other legal highs in prison. http://www.uservoice.org/wp-content/uploads/2016/05/User-Voice-Spice-The-Bird-Killer-Report-Low-Res.pdf. Accessed 11 Jun 2018

White JM, Irvine RJ (1999) Mechanisms of fatal opioid overdose. Addiction 94:961–972. https://doi.org/10.1046/j.1360-0443.1999.9479612.x

Wiley JL, Marusich JA, Thomas BF (2017) Combination chemistry: structure–activity relationships of novel psychoactive cannabinoids. In: Baumann MH, Glennon RA, Wiley JL (eds) Neuropharmacology of New Psychoactive Substances (NPS): the science behind the headlines. Springer, Cham, pp 231–248. https://doi.org/10.1007/7854_2016_17

Winstock AR, Barratt MJ (2013) The 12-month prevalence and nature of adverse experiences resulting in emergency medical presentations associated with the use of synthetic cannabinoid products. Hum Psychopharmacol 28:390–393. https://doi.org/10.1002/hup.2292

World Health Organization (WHO) (2015) International health regulations (2005) and chemical events. World Health Organization, Geneva

World Health Organization (WHO) (2018) International health regulations. http://www.who.int/topics/international_health_regulations/en/. Accessed 11 Jun 2018

Yeter O (2017) Identification of the synthetic cannabinoid 1-(4-cyanobutyl)-N-(2-phenylpropan-2-yl)-1H-indazole-3-carboxamide (CUMYL-4CN-BINACA) in plant material and quantification in post-mortem blood samples. J Anal Toxicol 41:720–728. https://doi.org/10.1093/jat/bkx061

Zaurova M, Hoffman RS, Vlahov D, Manini AF (2016) Clinical effects of synthetic cannabinoid receptor agonists compared with marijuana in emergency department patients with acute drug overdose. J Med Toxicol 12:335–340. https://doi.org/10.1007/s13181-016-0558-4

Emergence, Diversity, and Control of New Psychoactive Substances: A Global Perspective

Justice N. A. Tettey, Conor Crean, Susan C. Ifeagwu, and Martin Raithelhuber

Contents

Abstract

The phenomenon of new psychoactive substances (NPS), which came to the attention of the wider international community at the beginning of the 2010s, has been unprecedented in terms of the sheer number of substances, their rate of emergence, chemical diversity, and range of pharmacological effects. In particular, the chemical diversity has been a challenge to promoting a better understanding of the NPS market – a fundamental requirement for effective policy decisions and interventions. This manuscript highlights the significant chemical diversity of NPS and describes an alternative, complementary, and pragmatic classification based on pharmacological effects, which aligns NPS to traditional controlled drugs and enhances understanding of the phenomenon. It further reviews actions taken at the international level to address the NPS issue, including changes in the scope of control of some NPS and the enhancement of the United Nations Early Warning Advisory on NPS to deal with the dynamics and evolution of the market.

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Early warning advisory · New psychoactive substances · Scheduling

Acronyms of the Discussed New Psychoactive Substances (NPS)

25B-NBOMe	2-(4-Bromo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl) ethan-1-amine
25C-NBOMe	2-(4-Chloro-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl) ethan-1-amine
25I-NBOMe	2-(4-Iodo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl) ethan-1-amine
4,4′-DMAR	4-Methyl-5-(*p*-tolyl)-4,5-dihydrooxazol-2-amine
4Br-alpha-PVP	1-(4-Bromophenyl)-2-(pyrrolidin-1-yl)pentan-1-one
4Cl-alpha-PVP	1-(4-Chlorophenyl)-2-(pyrrolidin-1-yl)pentan-1-one
4F-alpha-PVP	1-(4-Fluorophenyl)-2-(pyrrolidin-1-yl)pentan-1-one
4-MEC	2-Ethylamino-1-(4-methylphenyl)propan-1-one
5F-APINACA	*N*-(Adamantan-1-yl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide
Acetylfentanyl	*N*-(1-Phenethylpiperidin-4-yl)-*N*-phenylacetamide
AH-7921	3,4-Dichloro-*N*-((1-(dimethylamino)cyclohexyl)methyl) benzamide
alpha-PVP	1-Phenyl-2-(pyrrolidin-1-yl)pentan-1-one
AM-2201	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](naphthalen-1-yl) methanone
APINACA (AKB-48)	*N*-(Adamantan-1-yl)-1-pentyl-1*H*-indazole-3-carboxamide
Benzylpiperazine	1-(Phenylmethyl)piperazine
Butyrfentanyl	*N*-(1-Phenethylpiperidin-4-yl)-*N*-phenylbutyramide
CP-47,497	*rel*-2-((1*R*,3*S*)-3-Hydroxycyclohexyl)-5-(2-methyloctan-2-yl)phenol
Ethcathinone	2-(Ethylamino)-1-phenylpropan-1-one
Ethylone	1-(Benzo[*d*][1,3]dioxol-5-yl)-2-(ethylamino)propan-1-one
Ethylphenidate	Ethyl-2-phenyl-2-(piperidin-2-yl)acetate
JWH-018	Naphthalen-1-yl(1-pentyl-1*H*-indol-3-yl)methanone
MDMB-CHMICA	Methyl *N*-{[1-(cyclohexylmethyl)-1*H*-indol-3-yl]carbonyl}-3-methyl-L-valinate
MDPV	1-(1,3-Benzodioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one
Mephedrone	2-(Methylamino)-1-(*p*-tolyl)propan-1-one
Methiopropamine	*N*-Methyl-1-(thiophen-2-yl)propan-2-amine
Methoxetamine	2-(Ethylamino)-2-(3-methoxyphenyl)cyclohexanone
Methylone	1-(1,3-Benzodioxol-5-yl)-2-(methylamino)propan-1-one
MT-45	1-Cyclohexyl-4-(1,2-diphenylethyl)piperazine
Pentedrone	2-(Methylamino)-1-phenylpentan-1-one
PMMA	1-(4-Methoxyphenyl)-*N*-methylpropan-2-amine

U-47700	3,4-Dichloro-*N*-((1*S*,2*S*)-2-(dimethylamino)cyclohexyl)-*N*-methylbenzamide
UR-144	(1-Pentyl-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl) methanone
XLR-11	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone

1 NPS Emergence Trends

The emergence of new psychoactive substances (NPS) is a global phenomenon that has affected all regions of the world, with over 111 countries and territories reporting this emergence to the United Nations Office on Drugs and Crime (UNODC) by the end of 2017 (UNODC 2018a). Following year-on-year increases in the emergence of NPS on global markets between 2009 and 2016, the rate of their appearance seems to have stabilized (Fig. 1) with almost 800 NPS reported by the end of 2017. Notwithstanding the apparent stabilization of the total numbers of NPS reported annually, the phenomenon is still dynamic with new substances emerging, some showing transience on the market, while others have been persistent since monitoring began in 2009. For example, in 2016, 72 NPS were reported for the first time, a much smaller number compared with 2015 (137) (UNODC 2018a). However, 70 of the 130 NPS

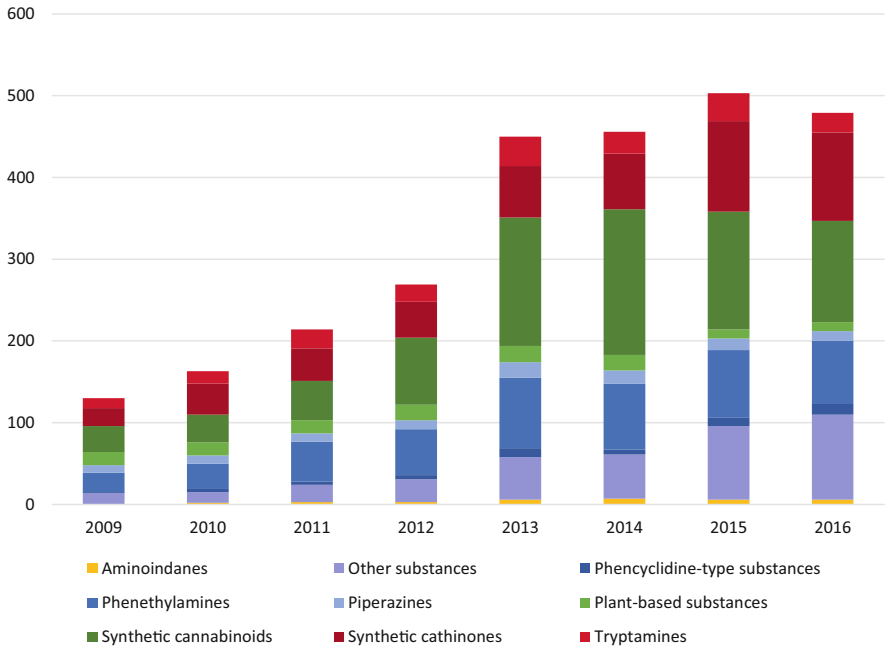

Fig. 1 NPS trend data. Source: UNODC Early Warning Advisory on NPS

identified at the start of monitoring in 2009 continue to be reported every year to this date. Following recommendations made by the World Health Organization (WHO), the Commission on Narcotic Drugs (CND) began to place several of these "persistent" NPS under international control in 2015, 2016, and 2017. In March 2018, the CND considered a further recommendation and placed 12 NPS, including 6 analogs of fentanyl, under control.

2 Understanding NPS: Chemical Versus Effect-Based Classification

Globally, the NPS market continues to be characterized by the appearance of large numbers of new substances of diverse chemistry. At the onset of NPS monitoring by the UNODC Early Warning Advisory (EWA), the dominance of a few chemical groups, such as the synthetic cathinones, phenethylamines, piperazines, phencyclidine-type substances, aminoindanes, and tryptamines, enabled classification based on chemical structures. This structural classification offered a number of benefits, including facilitating the development of analytical methods for the identification and characterization of substances within a particular group (UNODC 2017a) and providing the scientific basis for the design of national generic and analog legislation on NPS to address proliferation of substances in a defined chemical class. For example, grouping these substances in terms of chemistry enabled the adaption of the UNODC recommended methods of analysis of drugs series to the piperazines (UNODC 2013a), synthetic cathinones (UNODC 2015), synthetic cannabinoids (UNODC 2013b), and analogs of fentanyl (UNODC 2017b).

The diversity of NPS chemistry has continued to increase, and by 2017, the category of "other substances" monitored by the UNODC EWA had increased from 28 substances in 2012 to 155, representing 19% of all NPS reported to the system (Fig. 1). These are substances, which do not belong to the major chemical classes. Thirty-two new substances from this group, which emerged in 2015, were benzodiazepines, analogs of methylphenidate, and synthetic opioids related to fentanyl. This trend continued in 2016, with the emergence of several benzodiazepines and most notably 11 novel fentanyl derivatives (UNODC 2017c, d). In addition, several chemically unrelated substances with similar effects, such as stimulants related to phenmetrazine and hallucinogenic derivatives of lysergide (LSD), have been identified within the "other substances" category. This immense diversity provides a challenge to understanding the NPS phenomenon and synthetic drugs in general – a widely recognized, necessary step in developing effective responses at the global level (United Nations 2013).

The increase in chemical diversity over the years has not been limited to the category of "other substances" but evidenced in other major categories such as the synthetic cannabinoid receptor agonists (SCRAs) and synthetic cathinones.

2.1 Chemical Diversity of Synthetic Cannabinoids and Cathinones

Synthetic cannabinoid receptor agonists (SCRAs) represent over 30% of all NPS reported to the UNODC EWA. Up until 2012, the SCRAs reported were mostly either "classical" or "nonclassical cannabinoids," with substances related to tetrahydrocannabinol (e.g., CP-47,497) or aminoalkyl indoles such as JWH-018 or AM-2201. Generic legislation adopted by a number of countries sought to address possible substitutions of these defined core molecules. For example, the United Kingdom's generic legislation on NPS (The Misuse of Drugs Act 1971 [Amendment] Order 2009) targeted compounds structurally derived from 3-(1-naphthoyl) indole or 1*H*-indol-3-yl-(1-naphthyl)methane. This legislation specified substitution at the nitrogen atom of the indole ring by alkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl, or 2-(4-morpholinyl)ethyl, whether or not further substituted in the indole ring to any extent and whether or not substituted in the naphthyl ring to any extent.

While variations of this legislative language encompassed the majority of SCRAs reported globally over the period of 2009–2012, substances that emerged thereafter contained molecular features that bypassed the definitions used in generic legislation. These notably included the indazole carboxamides, *N*-(1-adamantyl)-1-pentyl-1H-*in*d*a*zole-3-*ca*rboxamide known as APINACA or AKB-48, and (1-pentyl-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone known as UR-144 (and its fluorinated derivative known as XLR-11). APINACA itself contains three molecular features, each of which, if present, would have allowed it to bypass the language used in existing generic legislation at that time. While the chemical modifications circumvented existing legislation, the understanding of these modifications on the extent of pharmacological actions of the resulting SCRA is at best sparse, especially in cases where novel substances emerge that have no published history in the scientific literature.

The period 2012–2014 marked a watershed moment in the proliferation of SCRAs with an overall increase of 78% in the number of substances reported (i.e., from 82 to 178). A number of derivatives that emerged during this time contained halogen atoms such as fluorine or chlorine, but most notably, 27 new substances containing carboxamide functional groups and 19 substances containing indazole ring systems (both present in APINACA) were reported to the UNODC EWA. Since 2015, the number of new SCRAs reported each year has decreased. However, with almost 250 SCRAs reported globally to date, the number of substances in this distinct pharmacological class is almost equivalent to the total number of all NPS that had emerged by 2013.

The synthetic cathinones represent another chemical class, which has experienced a significant increase in diversity in recent years, with 150 different substances reported to date. Substances within this group frequently affect the levels and actions of the monoamine neurotransmitters dopamine, norepinephrine, and serotonin, thus eliciting predominantly psychostimulant effects. Similar to a trend observed with the SCRAs in 2013, the increase in diversity of synthetic cathinones has often included

halogenated derivatives of substances reported previously. The single largest increase in the number of new synthetic cathinones was noted in 2015 (38 new substances), of which 34% were halogenated derivatives of previously reported substances. Notable examples also included the emergence of the alpha-PVP derivatives, 4F-alpha-PVP in 2013 and 4Cl-alpha-PVP and 4Br-alpha-PVP in 2015; ethcathinone derivatives carrying chlorine and bromine substituents at both the 3′ and 4′ positions were reported in 2015.

2.2 Effect-Based Classification of NPS

The structural classification of NPS has offered a number of benefits in terms of the development of analytical methodologies and formulation of analog and generic-based legislation, notwithstanding the complexities arising from proliferation of substances and groups. However, chemical similarity does not necessarily equate to similarity in pharmacological action. For example, the structurally similar buprenorphine ((5α,6β,14β,18*R*)-17-(cyclopropylmethyl)-18-[(2*S*)-2-hydroxy-3,3- dimethyl-2-butanyl]-6-methoxy-18,19-dihydro-4,5-epoxy-6,14-ethenomorphinan-3-ol) and diprenorphine ((5α,6β,14β,18*R*)-17-(cyclopropylmethyl)-18-(2-hydroxy-2-propanyl)-6-methoxy-18,19-dihydro-4,5-epoxy-6,14-ethenomorphinan-3-ol) differ only by three additional methyl groups on the same carbon atom (*tert*-butyl compared to methyl). However, while the former is an opioid receptor agonist, the latter is an opioid antagonist. Subsequently, the classification of NPS in terms of chemistry alone may not always facilitate an understanding of the drug market.

The review of substances for control under the international drug conventions requires evaluation of a wide range of scientific information, including chemistry, pharmacology, and toxicology. This allows a good correlation of chemistry to pharmacological effects. In contrast, a correlation between chemistry and pharmacological effects of NPS may not always be possible due to the paucity of scientific studies or literature on these substances. At the UNODC-WHO Expert Consultation on New Psychoactive Substances held in Vienna in December 2014 (CND 2015), experts recognized the challenges posed by the lack of scientific information on some NPS. Experts noted the usefulness of nonconventional sources of information, such as the Internet (specifically social media and Internet-based drug-user forums), as a tool to understand the NPS market, e.g., the effects and harms of substances, while also cautioning about its limitations, such as inter-individual variations.

The substances controlled under the international drug conventions produce psychoactive effects through a handful of pharmacological mechanisms. These include interactions with the opioid receptor and inhibitory neurotransmitters, activation of the cannabinoid receptor type 1 (CB_1), and the action of the neurotransmitter gamma-aminobutyric acid (GABA) at the $GABA_A$ receptor to produce, for example, sedative, hypnotic, and anxiolytic effects. Other substances have the capacity to modulate the levels and actions of the monoamine neurotransmitters dopamine, epinephrine, and serotonin, inducing a range of excitatory responses in the central nervous system and action as an *N*-methyl-D-aspartate (NMDA) receptor antagonist (United Nations 2016a).

This logic allows the assignment of all the substances controlled as of the end of 2017 under the international drug conventions, into six main "effect" groups (Fig. 2). These are the opioids (e.g., heroin and morphine), cannabinoid receptor agonists (e.g., cannabis and JWH-018), dissociatives (e.g., phencyclidine and methoxetamine), classic (serotonergic) hallucinogens (e.g., LSD and 2C-B), sedatives/hypnotics (e.g., diazepam), and psychostimulants (e.g., cocaine and amphetamine-type stimulants). It is worthy of note that some substances may produce effects resulting from more than one of the aforementioned groups. For example, the dissociative effects of phencyclidine as an NMDA receptor antagonist are also influenced by its uptake inhibition of dopamine, epinephrine, and serotonin (United Nations 2016a).

While NPS reported to date show significant diversity in chemistry, the major effects produced by most of these substances are available in the scientific literature and/or through nonconventional sources such as Internet-based drug-user forums. Figure 2 illustrates the distribution of substances under international control and of NPS in terms of their biological effects. While more than 68% of the substances under international control are synthetic opioids and sedatives/hypnotics, the distribution of NPS is more diverse, particularly with regard to classic hallucinogens, synthetic cannabinoid receptor agonists, and stimulants. Employing an effect-based classification of the NPS reported to date provides a matrix, which allows a facile alignment to the traditional controlled drugs and fosters a better assessment and understanding of the overall drug market. That almost 5% of all NPS reported have not yet been assigned in terms of the six effect-based groups illustrates the paucity of information on the actions of some of these substances and the unreliability of some self-reported user experiences and underlines the need for more research to establish their pharmacological effects. This notwithstanding, the pharmacological classification provides an alternative, complementary, and pragmatic system compared to the current system (Fig. 1) and should help in improving our understanding of the synthetic drugs market which is challenged by growing chemical diversity.

3 International Response to NPS

Efforts at the international level to address the issue of NPS have been comprehensive and wide ranging with the Commission of Narcotic Drugs (CND), a subsidiary body and a functioning commission of the Economic and Social Council (ECOSOC), providing leadership and direction on a myriad of responses and making decisions on the scope of control of NPS subject to investigation. Coinciding with almost 6 years of international action, and supported by a range of complementary efforts at national level, is the recent observation of the stabilization in the emergence of NPS globally.

CND Resolution 55/1, entitled "Promoting international cooperation in responding to the challenges posed by new psychoactive substances," marked the beginning of a series of multilateral actions and measures to address the NPS challenges (CND 2012). With over nine resolutions of the CND and the United

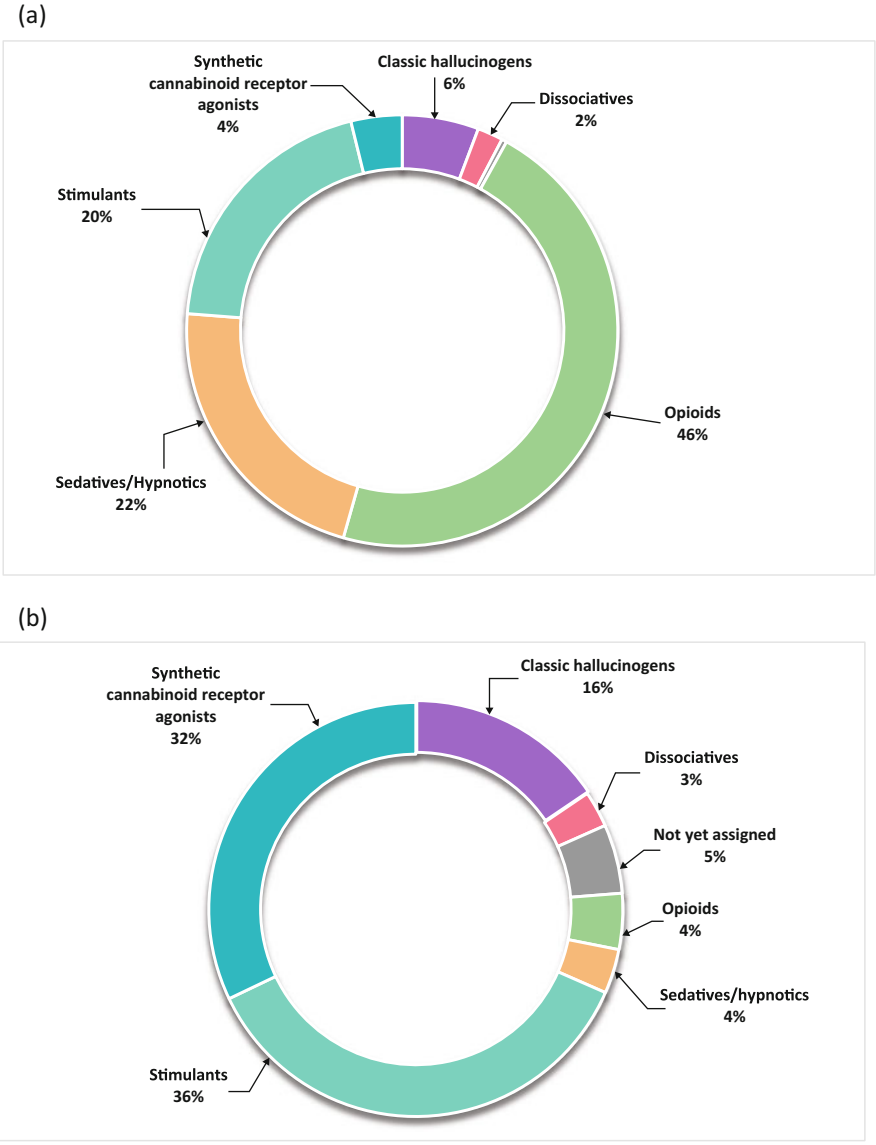

Fig. 2 Pharmacological effects of substances under international control and NPS. (**a**) Controlled substances by pharmacological effects ($n = 261$). (**b**) NPS by pharmacological effects. Source: UNODC Early Warning Advisory on NPS, December 2017 ($n = 779$). Note: The analysis of the pharmacological effects comprises NPS registered up to December 2017. Plant-based substances were excluded from the analysis as they usually contain a large number of different substances some of which may not have been known and whose effects and interactions are not fully understood

Nations General Assembly formulated between 2012 and 2017, finding a solution to the NPS phenomenon remains high on the agenda of the international community (Table 1).

Together, these resolutions have provided the basis for raising public awareness; monitoring, analysis, and sharing of NPS information and trends; international cooperation; and the development of early warning systems. In addition, these resolutions have encouraged both a public health approach to the NPS issue and the use of law enforcement interventions to reduce supply, legislative and administrative responses at the national level, and the review and scheduling of the most harmful, persistent, and prevalent NPS. Resolutions further called for the provision of technical assistance to affected countries in areas such as forensic capacity, to aid in the identification and detection of substances and in research, to improve the understanding of the phenomenon.

Table 1 Commission of Narcotic Drugs (CND) resolutions

Year	CND Resolution	Title/mandate
2010	CND resolution 53/7	International cooperation in countering the covert administration of psychoactive substances related to sexual assault and other criminal acts
2012	CND resolution 55/1	Promoting international cooperation in responding to the challenges posed by new psychoactive substances
2013	CND resolution 56/4	Enhancing international cooperation in the identification and reporting of new psychoactive substances
2014	CND resolution 57/9	Enhancing international cooperation in the identification and reporting of new psychoactive substances and incidents involving such substances
2015	CND resolution 58/3	Promoting the protection of children and young people, with particular reference to the illicit sale and purchase of internationally or nationally controlled substances and of new psychoactive substances via the Internet
2015	CND resolution 58/11	Promoting international cooperation in responding to new psychoactive substances and amphetamine-type stimulants, including methamphetamine
2016	CND resolution 59/8	Promotion of measures to target new psychoactive substances and amphetamine-type stimulants
2016	UNGASS outcome document A/S-30/L.1	Our joint commitment to effectively addressing and countering the world drug problem Operational recommendations on cross-cutting issues in addressing and countering the world drug problem: evolving reality, trends and existing circumstances, emerging and persistent challenges and threats, including new psychoactive substances, in conformity with the three international drug control conventions and other relevant international instruments
2017	CND resolution 60/4	Preventing and responding to the adverse health consequences and risks associated with the use of new psychoactive substances

3.1 Changes in the Scope of Control of Substances

The Single Convention on Narcotic Drugs of 1961, as amended by the 1972 Protocol (1961 Convention), the Convention on Psychotropic Substances of 1971 (1971 Convention), and the Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances of 1988 provide the basis for the international control of narcotics, psychotropic substances, and their precursors, respectively. These treaties ensure the availability of narcotic drugs and psychotropic substances for medical and scientific purposes while preventing abuse and harms due to their use, in order to protect human health and welfare. Enshrined in these treaties are provisions for changing the scope of control of substances, which is decided upon by the CND, upon the recommendation of the World Health Organization. Following the scheduling decisions of the CND, member state parties to these conventions are required to ensure that the mandatory control measures are applied to these substances (UNODC 2013c).

The dynamics of the NPS market, particularly their rapid appearance at the peak of the phenomenon identified in 2013, provided a challenge to the regular timelines for changing the scope of control of substances by the CND. This unprecedented state of rapid emergence triggered for the first time in the history of international drug control the use of provisional temporary control measures under Article 2(3) of the 1971 Convention. This provision is designed to prevent widespread abuse of a substance, in situations of urgency, before placing it under international control. In response to the rapid emergence and associated health harms of mephedrone, provisional control measures were implemented in 2014. Provisional control measures under the 1971 Convention are discretionary, and therefore member state parties to the convention are not bound to impose such measures (CND 2014). However, this provided a measure of control in some countries until the formal decision was made to place mephedrone under Schedule II of the 1971 Convention in 2015. Since the initial scheduling of an NPS in 2015, 27 substances have since been placed under various schedules of the 1961 and 1971 Conventions (UNODC 2018b).

Schedule I of the 1961 Convention, which contains substances that are highly addictive and liable to abuse (e.g., cannabis and heroin) or are convertible into drugs that are similarly addictive and liable to abuse, has been applied to the synthetic opioids AH-7921, acetylfentanyl, butyrfentanyl, MT-45, and U-47700. In addition, acetylfentanyl is subject to Schedule IV status under the 1961 Convention – a category for drugs listed in Schedule I that are highly addictive and liable to abuse and rarely used in medical practice.

The hallucinogenic phenethylamines 25B-NBOMe, 25C-NBOMe, and 25I-NBOMe and the psychostimulant PMMA are controlled under Schedule I of the 1971 Convention. This Schedule contains substances such as LSD, MDMA, and mescaline, which present a high risk of abuse, pose a particularly serious threat to public health, and are of very little or no therapeutic value. A large number of recently controlled substances have been placed in Schedule II of the 1971 Convention, which caters for substances such as the amphetamine-type stimulants (ATS)

and Δ^9-THC, which present a risk of abuse, pose a serious threat to public health, and are of low or moderate therapeutic value. The affected substances include the psychostimulants mephedrone, benzylpiperazine, MDPV, methylone, α-PVP, 4,4′-DMAR, 4-MEC, ethylone, pentedrone, ethylphenidate, and methiopropamine; the SCRAs JWH-018, AM-2201, MDMB-CHMICA, 5F-APINACA, and XLR-11; and the phencyclidine-type dissociative methoxetamine. In total, the twenty-seven substances that have been placed under international control since 2015 correspond to almost 20% of all reports of NPS to the UNODC EWA.

The period 1984–1990, which coincided with the emergence of several benzodiazepines and ATS, resulted in the number of substances controlled under the 1961 and 1971 Conventions increasing by 71. On this basis, a cursory look at the number of NPS controlled since the phenomenon came to the attention of the international community (i.e., 27 as at 2017), in relation to the high numbers that have emerged (almost 800 as at December 2017), might suggest a disproportionate response at controlling these substances at the international level. The 1961 Convention and the 1971 Convention delineate a clear role for WHO through its Expert Committee on Drug Dependence (ECDD) for carrying out risk assessments of substances based on medical and scientific evaluations as well as for issuing recommendations to the CND on changes in the scope of control of substances, where appropriate. The 36th Meeting of the ECDD held in 2014 represented the first time that the international system has had to deal with the phenomenon of NPS. The challenges identified by the WHO ECDD in the assessment of NPS included the lack of sufficient evidence required to recommend international control, especially for the assessment of dependence potential, abuse liability, and both individual and public health harm. In addition, the ECDD noted that limited systematic studies on NPS exist with most information only available from case reports (CND 2015). Such circumstances, coupled with the fact that scheduling under the international drug control conventions is done on a substance-by-substance basis, as opposed to the additional flexibility of generic and analog legislations at national level, might account for the relatively low proportion of controlled NPS.

To complement efforts at the international level, several member states have adopted a wide range of legislative measures to control NPS that are a threat to human health and well-being. Based on information provided by 59 member states to UNODC in 2015, several countries have adopted more than one type of legislation (UNODC 2016c). Most member states adopt the individual listing of substances under national legislation (Fig. 3). This has the advantage, in principle, that there is no ambiguity about whether or not a substance is covered by the control measures. Yet, a major drawback of this approach is that adding substance by substance to the schedules of national drug laws can become a lengthy procedure, which may not provide a fitting response to the fast-paced nature of the NPS market. A number of countries have adopted different types of legislation in response to the unprecedented proliferation of substances. These include legislation that allows the simultaneous control of clusters of substances, known as generic control. In practice, such a legislative approach defines specific variations, of a core molecular structure, which are controlled. In this way, synthetic substances can be controlled without being specifically referred to in the legislation by invoking the concept of “chemical

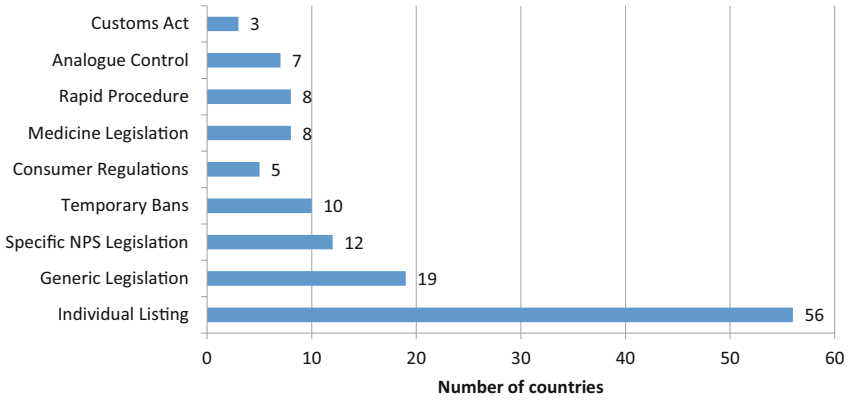

Fig. 3 National legislative approaches by number of countries (based on information provided by 59 countries). Source: Global SMART Update Volume 16. Post-UNGASS 2016: NPS trends, challenges, and recommendations

similarity" to a drug that is already controlled. Other countries have experimented with analog controls, which are much broader than generic controls since they address more general aspects of similarity in chemical structure to a "parent" compound.

However, the rapid proliferation of NPS on the market and the diversity of substances have prompted some governments to look for alternatives to existing drug control systems that are solely based on the chemistry of substances. For example, in the case of the United States, the Synthetic Drug Abuse Prevention Act of 2012 for the first time introduced controls of synthetic cannabinoids, not only based on their chemical nature but also referring to the effects on the brain according to a "neurochemical definition." Psychoactive Substances Acts, such as the one introduced in the United Kingdom, also underline this transition from chemistry to psychoactive effects as a basis for control in a rapidly evolving synthetic drugs market (Psychoactive Substances Act 2016).

3.2 Early Warning Systems: From Awareness Raising to Addressing Threats

While the scope of issues covered by CND resolutions on NPS have been comprehensive (Table 1), they have also demonstrated flexibility in responding to the dynamic nature of the phenomenon (e.g., appearance, disappearance, and reemergence of substances) and the challenges resulting from the paucity of information to enable an evidenced-based scheduling of substances. Of significance to international actions is the recognition that not all NPS that have emerged on the global market satisfy the criteria for the risk of harm required for international control (UNODC 2016d). The evolution of the UNODC EWA, described below, provides a unique example of such a course chartered by flexible and dynamic decisions by the CND.

Early warning systems play a key role in monitoring, early detection, and timely responses to emerging threats. They also aim at providing the necessary evidence base to inform policies and responses to the identified threats. The Sustainable Development Goals (SDGs) of the 2030 Agenda recognize the role of early warning systems in achieving Goal 3 on good health and well-being (United Nations 2018). A number of countries and regions have adopted strategies to monitor NPS. The Early Warning System of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), implemented in 1997 as part of the Joint Action (97/396/JHA: Joint Action of 16 June 1997), represents a good example of best practice in terms of regional cooperation in the use of early warning.

In its Resolution 56/4 (March 2013) titled "Enhancing international cooperation in the identification and reporting of new psychoactive substances," the CND recognized the importance of sharing information on NPS at a global level (CND 2013). This led to the development and launch of the UNODC Early Warning Advisory (EWA) in March 2013. Currently, with over 16,000 data points on almost 800 NPS from 111 countries collected since 2008, including information on substances, country and year of emergence, and national legislative responses, the UNODC EWA provides a means of determining, through trend analysis, the global prevalence of a substance and also its market persistence, including disappearance, market stability, and post-legislative effects. It has also helped in establishing global trends of NPS and in identifying new and emerging threats. The EWA has also served member states and relevant international organizations, such as the WHO, in the identification and prioritization of candidate substances for scheduling notification and/or control.

Since its launch in 2013, the continuous evolution of the EWA reflects an incremental understanding of the key features of the NPS phenomenon, which include the threat to public health and safety, the unprecedented rate of emergence, diversity and heterogeneity with regard to types of substances, and extent of the problem in various regions (Fig. 4). Despite the high numbers of NPS reported to date, it is known that they are diverse in nature and pharmacological action (Dargan and Wood 2013). Some NPS are only transient in their existence on drug markets, and not all NPS that have emerged on the global market satisfy the criteria for the risk of harm required for international control (UNODC 2016d). Subsequently, the 2016 United Nations General Assembly Special Session (UNGASS) on the World Drug Problem was a landmark in the evolution of the EWA. Member states recognized the need for a comprehensive strategy to tackle harmful NPS and reinforced the need to prioritize "the most harmful, persistent and prevalent NPS for action" (United Nations 2016b, UNODC 2016c).

Toxicology data on NPS are vital to understanding the associated harms, and the knowledge gained by toxicologists is pivotal to informing early warning systems. In 2018, the EWA began to feature an online module for reporting information on the toxicology of NPS (Ifeagwu et al. 2017) with the objective of facilitating information sharing between the toxicology community and related stakeholders while supporting the prioritization of the most harmful, prevalent, and persistent NPS for international control (UNODC 2018c). The use of the EWA to prioritize

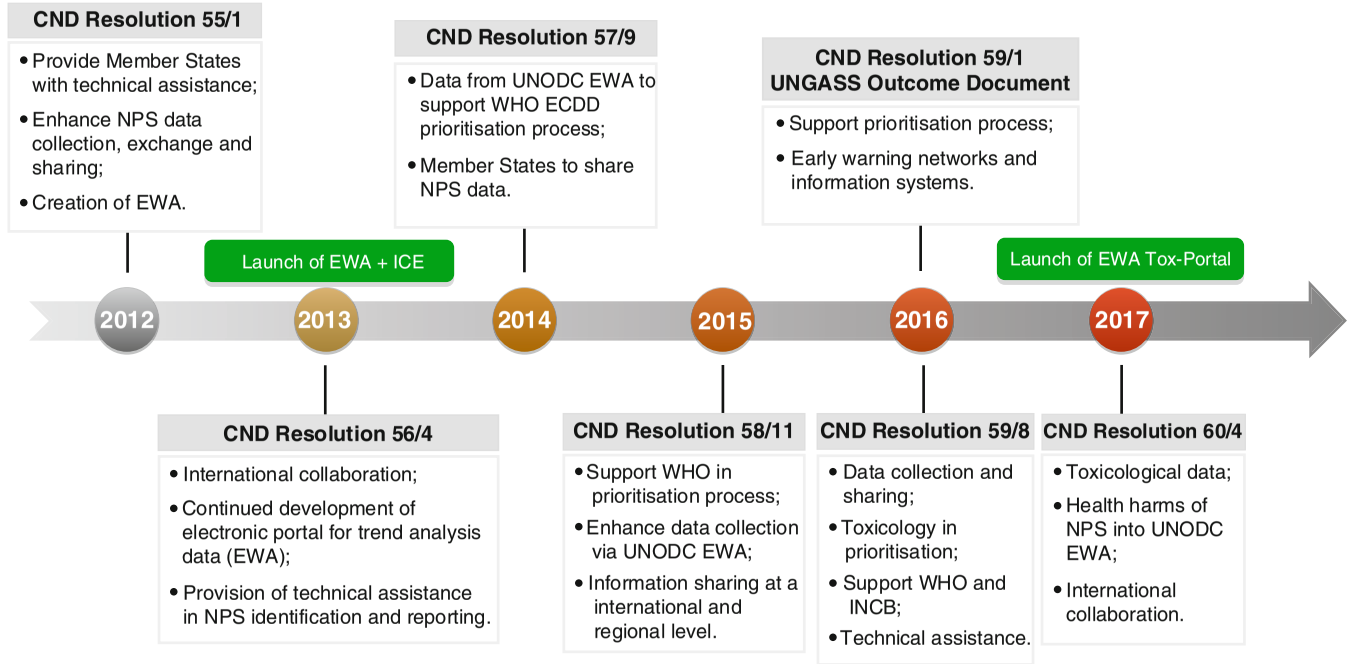

Fig. 4 Timeline of Commission on Narcotic Drugs (CND) resolutions from 2012 to 2017

NPS that require an international response will keep the international drug control system focused on the substances most harmful to humankind and support efforts in reducing supply and protecting health. In addition, an established network of over 235 national drug-testing laboratories in more than 70 countries, participating in the UNODC International Collaborative Exercises (ICE) Programme (UNODC 2018d) and directly linked to the EWA, helps raise awareness of the harmful NPS on the market. It further assists in the development of appropriate laboratory capacity for identification and detection of such NPS and subsequently supports the preparedness of countries to address the identified threats from both a supply reduction and demand reduction perspective.

4 Conclusions

Waves of emergence of large numbers of substances on drug markets are not new, and the episodes involving the appearance of fentanyl analogs, benzodiazepines, and amphetamine-type stimulants in the 1970s, 1980s, and 1990s illustrate this cyclic feature of the drug market. However, the NPS phenomenon, which came to the attention of the wider international community at the beginning of the 2010s, has been unprecedented in terms of the sheer number of substances, their rate of emergence, the chemical diversity, and the range of pharmacological effects. The chemical diversity has been a challenge to efforts to gain a better understanding of the market – a key to effective policy decisions and interventions. The unique nature of the NPS phenomenon has required innovative approaches. Over the period 2012–2017, multilateral efforts through the Commission on Narcotic Drugs have produced responses to the NPS, which have been multifaceted, comprehensive, and balanced. A number of the most harmful, persistent, and prevalent NPS have been placed under international control, and current trends indicate a decrease in innovation, with fewer new substances emerging. The continuous development of the UNODC EWA since its inception in 2013 provides the international community with a tool for identifying NPS, anticipating threats, and ensuring the preparedness of member states to address these threats. In addition, the UNODC continues to support member states in the identification and detection of NPS – an essential requirement for any law enforcement or health intervention – through its scientific and forensic services program. However, challenges remain with the increase in the number of substances with opioid and sedative/hypnotic properties. Concerted efforts will continue to be required for the exchange of information and sharing of best practices, including early warning systems, to help member states make informed decisions and enhance their preparedness to address threats associated with NPS. A major future challenge would be an assessment as to how to maintain the efforts and to evaluate successful strategies in mitigating harms associated with the NPS problem and to find effective and prompt solutions in dealing with the dynamics and evolution of the NPS market.

References

97/396/JHA (1997) Joint Action of 16 June 1997 adopted by the Council on the basis of Article K.3 of the Treaty on European Union, concerning the information exchange, risk assessment and the control of new synthetic drugs. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31997F0396:EN:HTML. Accessed 27 Feb 2018

Commission on Narcotic Drugs, CND (2012) Report on the fifty-fifth session (13 December 2011 and 12–16 March 2012). http://undocs.org/E/2012/28-E/CN.7/2012/18

Commission on Narcotic Drugs, CND (2013) Resolution 56/4. Enhancing international cooperation in the identification and reporting of new psychoactive substances. http://undocs.org/E/2013/28-E/CN.7/2013/14

Commission on Narcotic Drugs, CND (2014) Challenges and future work in the review of substances for possible scheduling recommendations. Note by the Secretariat. http://undocs.org/E/CN.7/2014/10

Commission on Narcotic Drugs, CND (2015) UNODC-WHO expert consultation on new psychoactive substances, Vienna, 9–11 December 2014. Report by LSS/RAB/DPA/UN. http://undocs.org/E/CN.7/2015/CRP.2

Dargan P, Wood D (2013) Novel psychoactive substances: classification, pharmacology and toxicology. Academic, New York

Ifeagwu SC, Raithelhuber M, Crean C, Gerostamoulos D, Chung H, Tettey JN (2017) Toxicology in international drug control—Prioritizing the most harmful, persistent and prevalent substances. Forensic Sci Int 274:2–6. https://doi.org/10.1016/j.forsciint.2016.11.022

Psychoactive Substances Act (2016) http://www.legislation.gov.uk/ukpga/2016/2/contents/enacted. Accessed 15 Jan 2018

The Misuse of Drugs Act 1971 (Amendment) Order (2009) No. 3209 Article 2. https://www.legislation.gov.uk/uksi/2009/3209/article/2/made. Accessed 15 Jan 2018

United Nations (2013) The international drug control conventions. https://www.unodc.org/documents/commissions/CND/Int_Drug_Control_Conventions/Ebook/The_International_Drug_Control_Conventions_E.pdf. Accessed 22 Jan 2018

United Nations (2016a) Terminology and information on drugs. https://www.unodc.org/documents/scientific/Terminology_and_Information_on_Drugs-3rd_edition.pdf. Accessed 2 Feb 2018

United Nations (2016b) Our joint commitment to effectively addressing and countering the world drug problem. http://undocs.org/A/RES/S-30/1

United Nations (2018) Sustainable development goal 3. https://sustainabledevelopment.un.org/sdg3. Accessed 15 Jan 2018

UNODC (2013a) Recommended methods for the identification and analysis of Piperazines in Seized Materials. https://www.unodc.org/documents/scientific/STNAR47_Piperazines_Ebook.pdf. Accessed 2 Feb 2018

UNODC (2013b) Recommended methods for the identification and analysis of synthetic cannabinoid receptor agonists in seized materials. https://www.unodc.org/documents/scientific/STNAR48_Synthetic_Cannabinoids_ENG.pdf. Accessed 2 Feb 2018

UNODC (2013c) UNODC Global SMART Programme. The challenge of new psychoactive substances. https://www.unodc.org/documents/scientific/NPS_Report.pdf. Accessed 16 Jan 2018

UNODC (2015) Recommended methods for the Identification and Analysis of Synthetic Cathinones in Seized Materials. https://www.unodc.org/documents/scientific/STNAR49_Synthetic_Cathinones_E.pdf. Accessed 2 Feb 2018

UNODC (2016c) Global SMART Update Volume 16. Post-UNGASS .2016: nps trends, challenges, and recommendations. https://www.unodc.org/documents/scientific/Global-SMART-Update-2016-vol-16.pdf. Accessed 15 Jan 2018

UNODC (2016d) New psychoactive substances: overview of trends, challenges and legal approaches. http://undocs.org/E/CN.7/2016/CRP.2

UNODC (2017a) Publications. https://www.unodc.org/unodc/en/scientists/publications-drug-testing-laboratories.html. Accessed 30 Jan 2018

UNODC (2017b) Recommended methods for the identification and analysis of fentanyl and its analogues in biological specimens. https://www.unodc.org/documents/scientific/Recommended_methods_for_the_identification_and_analysis_of_Fentanyl.pdf. Accessed 2 Feb 2018

UNODC (2017c) Global SMART Update Volume 17. Fentanyl and its analogues - 50 years on. https://www.unodc.org/documents/scientific/Global_SMART_Update_17_web.pdf. Accessed 16 Jan 2018

UNODC (2017d) Global SMART Update Volume 18. Non-medical use of benzodiazepines: a growing threat to public health? https://www.unodc.org/documents/scientific/Global_SMART_Update_2017_Vol_18.pdf. Accessed 16 Jan 2018

UNODC (2018a) Early warning advisory on NPS. http://www.unodc.org/nps. Accessed 30 Jan 2018

UNODC (2018b) International drug control conventions. https://www.unodc.org/unodc/en/commissions/CND/conventions.html. Accessed 31 Jan 2018

UNODC (2018c) Early warning advisory toxicology portal. http://www.unodc.org/tox. Accessed 15 Jan 2018

UNODC (2018d) International Collaborative Exercises (ICE) programme. http://www.unodc.org/ice. Accessed 15 Jan 2018

Self-Experiments with Psychoactive Substances: A Historical Perspective

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Abstract

The purpose of this chapter is to highlight the rich tradition of self-experiments (SEs) with psychoactive substances carried out by scientists and therapists for more than a century. Scientifically inspired controlled SEs dominated until the end of the twentieth century, when ethical requirements minimized controlled SEs and "wild" SEs expanded particularly with the emergence of new psychoactive substances. The review focuses on laughing gas (nitrous oxide), cannabis, cocaine, hallucinogens, entactogens, and dissociative hallucinogens. This is due to the fact that substances that induce "complex" effects such as alteration of space/time experience, ego dissolution, and increased feelings and insights (e.g., hallucinogens, entactogens) represent by far the majority of SEs, whereas SEs with substances inducing "simple" effects such as euphoria, anxiolysis, dissociation, or emotional blunting (e.g., cocaine, opioids) are much rarer or even absent (e.g., benzodiazepines). Complex drug effects are much harder to describe, thus allowing SEs to fulfill a more important function.

SEs with psychoactive drugs appeared to emerge in the mid-eighteenth century, which triggered a long-standing tradition throughout the nineteenth and early twentieth century. SEs have been de facto performed for a variety of reasons, ranging from establishing scientific knowledge and gaining philosophical insights to compensating for personal deficits. Self-experimenters can be divided into two general types. Besides their scientific intentions, "exploratory" self-experimenters intend to expand awareness and insight, whereas "compensatory" self-experimenters might aim for coping with psychiatric symptoms or personality deficits. Scientific limitations of SEs are obvious when compared to double-blind, randomized, placebo-controlled trials. Whereas the former might lead to more "realistic" detailed description of subjective effects, the latter lead to more solid results in respect to objectively measurable "average" effects. Possible adverse effects of SEs were identified that resulted in loss of scientific objectivity and decreased control over substance use and addiction, development of isolation, problematic group dynamics, and "social autism."

Keywords

Cannabis · Cocaine · Dissociative hallucinogens · Entactogens · Hallucinogens · History of drug use · Nitrous oxide · Psychoactive substances · Self-experiments

Acronyms of the Discussed Psychoactive Substances

2C-B	2-(4-Bromo-2,5-dimethoxyphenyl)ethan-1-amine
2C-E	2-(4-Ethyl-2,5-dimethoxyphenyl)ethan-1-amine
2C-T-2	2-[4-(Ethylsulfanyl)-2,5-dimethoxyphenyl]ethan-1-amine
2C-T-7	2-[2,5-Dimethoxy-4-(propylsulfanyl)phenyl]ethan-1-amine
2C-T-4	2-[2,5-Dimethoxy-4-(propan-2-ylsulfanyl)phenyl]ethan-1-amine
2C-T-21	2-{4-[(2-Fluoroethyl)sulfanyl]-2,5-dimethoxyphenyl}ethan-1-amine
5-HO-DMT	3-[2-(Dimethylamino)ethyl]-1*H*-indol-5-ol
AM-2201	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](naphthalen-1-yl)methanone

LSD	(8β)-*N*,*N*-Diethyl-6-methyl-9,10-didehydroergoline-8-carboxamide (d-lysergic acid diethylamide)
MDA	1-(2*H*-1,3-Benzodioxol-5-yl)propan-2-amine
MDE	1-(2*H*-1,3-Benzodioxol-5-yl)-*N*-ethylpropan-2-amine
MDMA	1-(2*H*-1,3-Benzodioxol-5-yl)-*N*-methylpropan-2-amine
MEM	1-(4-Ethoxy-2,5-dimethoxyphenyl)propan-2-amine
MPPP	1-Methyl-4-phenylpiperidin-4-yl propanoate
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

1 Introduction

Since ancient times, it seemed like a usual behavior of humans, and especially so with physicians and scientific researchers, to conduct experiments not only on other humans (volunteers or "paid participants") but also on themselves. From a historical perspective, it seemed appropriate for a long time to conduct experiments on fellow human beings for the purpose of saving lives, for facilitating cure and healing, and/or for providing other benefits. An old principle of medical morality is to never perform an experiment on man if it might turn out to be harmful to any extent.

The ancient Greeks and Romans did not appear to experiment much on healthy or ill people. Hippocrates, the ancient medical philosopher, has even warned doctors to experiment with new and unknown techniques or drugs. This attitude may have contributed to the fact that progress in the medical sciences was put on hold for hundreds of years. Since the medieval ages, medical and other investigators began to experiment on fellow humans and on themselves with the aim of making new discoveries about the human organism and for the purpose of improving treatment options.

The Oxford English Dictionary does not contain the term self-experimentation or auto-experimentation, but it includes the term self-experience. This is traced back to 1778, when it was defined as "those that have self-experience, are usually more affected than those that have things by hear-say only" (Simpson and Weiner 1989).

Self-experiments (SEs) are experiments in which physicians, psychologists, or other researchers serve as their own experimental subjects. The French physiologist Claude Bernard (1813–1878) emphasized the importance of such experimentation: "Morals do not forbid making experiments on one's neighbor or on one's self. . . . Christian morals forbid only one thing, doing ill to one's neighbor. So among the experiments that may be tried on man, those that can only harm are forbidden, and those that may do good are obligatory" (Castiglioni 1947, p. 598).

After World War II, it emerged that such principles were found to be seriously defiled by Nazi doctors in Germany. However, during that time there was no formal code of ethics in medical research to which the judges at the Nuremberg trials could rely on. As a result, the *Nuremberg Code for medical experiments* was established. It was especially found necessary to obtain informed consent from participating subjects for any type of experiment. In respect to self-experimentation, paragraph 5 of this Code includes the following formulation: "No experiment should be

conducted where there is an a priori reason to believe that death or disabling injury will occur; except, perhaps, in those experiments where the experimental physicians serve as subjects" (Nuremberg Code, quoted in Altman 1986, p. 17). This points toward somewhat lower safety standards with regard to SEs when performed by physicians or other scientists.

2 Self-Experimentation in Medicine

In the eighteenth and nineteenth century, a broader discussion on the issue of self-experimentation emerged. The general opinion prevailed that SEs were considered a requirement before administering any medication to a patient, which was also deemed applicable to other medical procedures such as vaccination or anesthesia. However, not every new medication or treatment was tested in SEs.

The topic of self-experimentation has received little attention in the scientific literature and in discussion surrounding codes of ethics. Altman (1972) found just 137 SEs documented in the medical literature. However, when it comes to SEs with psychoactive substances reviewed here, one has to expand this list significantly. There are more than 100 publications available that involve SEs carried out by medical and psychological researchers, and no bibliography appears to list these SEs.

Reasons for conducting SEs are manifold. Usually, SEs conducted by medical doctors include the following reasons: to observe, to assess therapeutic benefits to accumulate data, to study physiological processes, to explore mechanisms associated with the transfer of infections, to test newly developed instruments (e.g., cardiac catheter), to test instruments or medications for minimizing risks of harm to patients, and/or to explore resilience. In the field of psychoactive substance research, some investigators have repeatedly taken the burden of self-experimentation in an effort to explore the perspective of experimental subjects in order to optimize and refine the procedure and atmosphere needed for appropriate clinical experiments. At the same time, it is recognized that SEs provide only limited data when it comes to modern scientific standards. For example, the inclusion of double-blind, randomized, placebo-controlled trials would be needed to collect solid data on effects and risks associated with psychoactive substances. Nevertheless, in contrast to controlled SEs, more rigorously designed scientific experiments often fail to provide data about the "inner experiences" and more complex subjective effects elicited by psychoactive substances.

Henry K. Beecher, the first professor of anesthesia at Harvard University in Boston, wrote in 1959: "Experimentation upon other men requires a willingness to experiment on oneself as evidence of good faith . . ." (Beecher 1959). A statement made by Sir George Pickering points in the same direction: "The experimenter has one golden rule to guide him as to whether the experiment is justifiable. Is he prepared to submit himself to the procedure? If he is, and if the experiment is actually carried out on him, then it is probably justifiable. If he is not, then the experiment should not be done" (Pickering 1949). These statements imply that the

willingness to SE is a necessary requirement for any experimentation on other human beings. Leo Alexander, who was a major figure in writing the Nuremberg Code, differentiated this point of view: "It is ethically permissible for an experimenter to perform experiments involving significant risks only if the solution, after thorough exploration of all the other lines of … scientific investigation, is not accessible by any other means, and if he considers the solution of the problem important enough to risk his own life along with those of his non-scientific colleagues …" (quoted by Altman 1986, p. 17). However, some significant medical institutions like the National Institute of Health in the USA permit SEs just in those cases where "the same safeguards for the investigator-subject [were provided] as for a normal volunteer." One implication is that all SEs have to undergo a complete medical examination beforehand. The Johns Hopkins Hospital issued a memorandum in 1983, reminding their medical doctors that proposed SEs must be submitted for review in the same way as any investigation using human volunteers (Altman 1986, p. 20).

One prominent example involving SEs was John Scott Haldane (1860–1936). Haldane's experiments were mainly focused on studying the impact of gases on breathing using himself as the main volunteer. His aim was "to achieve knowledge, which could save other men's lives." Haldane argued that experimentation on animals was insufficient because it was conducted on anesthetized animals. Therefore, he started experimenting on himself and a close associate and gained groundbreaking results at the time (Haldane and Smith 1893; Haldane 1922). His studies were later referred to as the "most fundamental studies and far-reaching contributions to physiology" (Altman 1986, p. 217).

3 Self-Experimentation with Psychoactive Substances

In respect to psychoactive substances, it appears that the first documented systematic SEs have been published by Horace Wood (1869), a physician and professor of botany at the University of Pennsylvania, who won an American Philosophical Society price for his descriptions of SEs using an extract of the cannabis plant.

In 1896, Weir Mitchell, a pioneering American neurologist, began to carry out SEs with the mescaline-containing peyote cactus, which led to the first detailed description of its psychological effects (Weir Mitchell 1896). His experiment awakened a whole new strain of self-experimentation with psychoactive substances that resulted in a significant expansion since the turn to the twentieth century.

Because animal experiments are of rather limited value when it comes to assessing psychological effects, one would expect that research on hallucinogenic or psychedelic substances should have evoked significant self-experimentation by researchers, chemists, and therapists. This was confirmed by a large number of SEs documented in relation to hallucinogens, entactogens, and dissociative drugs, such as ketamine.

4 Defining the Topic

The purpose of this chapter is to highlight the rich tradition of self-experimentation in the field of psychoactive substance research that spans more than a century. For unknown reasons, the topic related to SEs with psychoactive substances, despite having a long-standing tradition in medical research contexts, has not been considered in the authoritative review on medical self-experimentation published by Altman (1986). Due to the large amount of material available, the scope of this chapter had to be limited in two ways:

1. Specific types of psychoactive substances that are considered to produce rather "simple" patterns of effects have been excluded, such as benzodiazepines, antidepressants, simple amphetamine-like stimulants, and (synthetic) opioids/opiates. These substances produce relatively easily predictable psychological effects. Specifically, the spectrum of internal experiences induced by these types of drugs shows a more uniform pattern with rather small interindividual variation (Table 1). It is also obvious from the existing literature that these substances were comparatively less frequently studied in SEs (Fig. 1). This is in contrast to hallucinogens, hashish, and the (somewhat more "complex") psychostimulant cocaine. These substances seem to produce "more interesting," complex, and challenging effects, which include large interindividual variation. Another reason for encountering more SEs with these types of substances might be associated with the eminently subjective character of the experience, which cannot be easily described to someone who has not experienced them.

Table 1 "Simple" and "complex" effects of psychoactive drugs[a]

"Simple" *drug effects*
Increase or decrease of arousal
Hypervigilance or clouding of consciousness
Euphoria
Anxiolysis, relaxation
Decrease of emotional reactivity, memory, self-perception
"Complex" *drug effects*
Pseudo-hallucination, hallucination, synesthesia
Enhanced visual imagery
Intensification of affectivity (euphoria, dysphoria, anxiety)
Alteration of space/time experience
Altered thought processes (less abstract, more imaginative, unusual associations)
Memory changes (hypermnesia, age regression)
Different degrees of ego-dissolution
Mystical-type experiences

[a]It has to be noted that psychoactive substances that induce "complex" effects do not typically induce one or two types of effects but usually involve more than five at the same time, which contributes to the complexity of the subjective effects experienced and reported

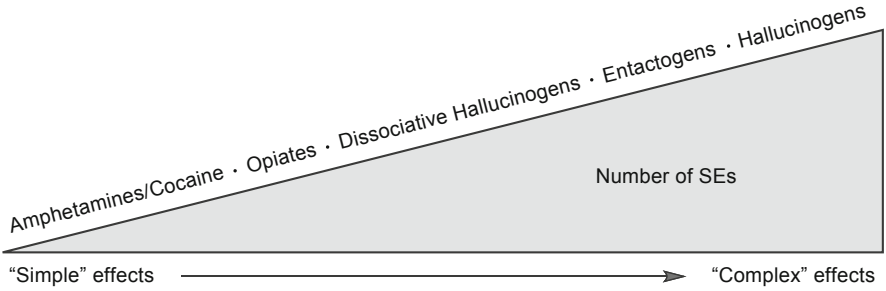

Fig. 1 Increasing complexity of drug effects (left to right) correlates with the amount of documented scientifically driven self-experiments (SEs)

2. Another limitation is that this review will be restricted to SEs performed by physicians, psychologists, or medicinal chemists. In general, information about other groups of researchers in the scientific arena who carried out SEs is rather limited. Clearly, there is an abundance of information available, most notably published by writers, intellectuals, and artists, but an inclusion of this aspect is beyond the scope of this chapter. However, a few examples outside these limits have been included.

4.1 Definitions of Self-Experiments with Psychoactive Substances

As far as self-experimentation with psychoactive substances is concerned, a classification into three types of SEs has been adopted for the purpose of this chapter:

4.1.1 Controlled Self-Experiments

These SEs are usually carried out in a controlled (clinical) environment, are seriously planned in advance, and are conducted with an exactly defined dose. Usually, some knowledge on the effects of the drug is provided in advance. They are usually intended to facilitate some form of systematic self-observation. In most cases, the subject is a physician or a scientist, and an outside observer and/or a person supervising the experiment is also present. Details about the experiment are documented and include information about dose, mode of application, environment, and experimental procedure. Usually, a written self-report is provided by the experimenter. The reasons for this kind of approach can differ. Sometimes, only a one-time test of a substance is of interest. On other occasions, it might be intended to learn more about the subjective experiential pattern induced by a specific substance. In other cases, there may be a desire to learn more about pathological conditions "from inside", for example, by inducing a "psychosis"-like state in the psychiatrist or psychotherapist himself. Most experiments reviewed in the present review chapter belong to this category.

4.1.2 Uncontrolled Self-Experiments

Uncontrolled SEs are those in which a scientist is at first testing a substance on himself, but without giving much detail and documentation on what was specifically done and experienced. Important parameters of the experiment such as its method and descriptions might not be controlled or even completely missed. An outside observer might be present or not. Specific measures or instruments to objectify some of the effects experienced are not used. Nevertheless, the testing might be considered useful for certain purposes, such as self-awareness, insights into the subject's reactions and observations about how to cope with the drug effect, etc. Uncontrolled self-experimentation however suffers from the possibility of obtaining irreproducible results. One potential concern is that uncontrolled SEs might lead to unrealistic and dangerous behavior either during or after the experiment. The subject might also be confronted with a disabled state of helplessness. Other risks might also involve stepping outside the scientific framework and methodology or getting "out of control," for example, by developing drug dependence and thus resulting in a problematic pattern of use and/or adverse psychosocial consequences. Nevertheless, in most cases these dangers appeared to be limited. At the same time, the type of drug used in most of the SEs needs to be considered. For example, hallucinogens and entactogens do not appear to display the dependence potential observed with cocaine or other euphoric psychostimulants (Nichols 2016).

4.1.3 "Wild" Self-Experiments

These are SEs, which are not intended to lead to scientifically relevant knowledge and are therefore, somewhat beyond the scope of this chapter. In this context, experimenters might come from a broad range of backgrounds including problematic drug users in search for another "high." From a historical perspective, "wild" SEs were not commonly encountered in the eighteenth and nineteenth century although some of the early experiments with nitrous oxide (laughing gas) may belong to this category. At the same time, this particular category represents a significant part of less systematically conducted self-experimentation known since the 1960s. Usually, these experiments are not planned as much in advance. They are not primarily undertaken to gain scientific data in the traditional sense. A primary motive can be the testing of a new drug unknown to the person or a testing of purity and dose. Motivations range from curiosity about testing newly appearing substances "for the health of the drug user community" (Soussan and Kjellgren 2014) to a more serious scientific or therapeutic interest and self-treatment. This does not exclude the possibility that the experimenter has undergone significant preparation and that the circumstances under which the experiment is conducted are carried out in a safe and serious fashion.

5 Self-Experimentation with New Psychoactive Substances (NPS)

From the mid-1960s onward, "wild" drug self-experimentation carried out by laymen became a mass phenomenon. However, the earliest origins can be traced back to the 1950s, when some artists and writers began to experiment with drugs, who later became known as "beatniks" (Bisbort 2009). In the early 1960s, "wild" SEs also began to appear in therapeutic and research circles in the USA. A much wider definition of "wild" SEs might include lay use of these substances since the mid-1960s when millions of young people in the USA and elsewhere ingested hallucinogens such as LSD, mescaline, or psilocybin.

During the last four decades, a process of cultural adaption to these drugs has taken place (Henderson and Glass 1994). Legislation, adapted medical treatment, as well as harm reduction through informal learning processes of user groups are placed in this perspective. These developments have limited the distribution of these drugs but also established a "black" market. Due to legislative control of the classic stimulants, hallucinogens, entactogens, and dissociative hallucinogens, drug producers and distributors were eager to develop substances that did not fall under control measures. Eventually, this intention led to the emergence of so-called designer drugs in the early 1980s. MDMA as well as a range of other substances (e.g., synthetic opioids, piperazines, or phencyclidine-based compounds) were examples of these early attempts to circumvent the law (Kirsch 1986; Passie and Benzenhöfer 2016; Henderson 1988; Morris and Wallach 2014).

Since the publication of Shulgin and Shulgin's PIHKAL (Shulgin and Shulgin 1991) and TIHKAL (Shulgin and Shulgin 1997), many new substances started entering the recreational drug market (e.g., King and Kicman 2011; King 2014). In the late 1990s, a new and more organized market of "party pills" and "research chemicals" began to emerge, which was intended to provide uncontrolled alternatives. At this moment, the umbrella term "NPS" typically tends to refer to substances that are not controlled internationally but that may pose comparable threats to public health, which means that they are therefore not listed in any of the Schedules of the United Nations' drug control conventions (Brandt et al. 2014; Evans-Brown and Sedefov 2018). From a practical perspective however (e.g., data collection and monitoring), substances that have been placed under international control more recently still tend to be viewed as NPS. The number of NPS detected globally reached about 800 by the end of 2017, and the diversity of drug classes has increased within the last 10 years (Tettey et al. 2018). Commonly encountered drug classes include psychostimulants, synthetic cannabinoid receptor agonists, entactogens, hallucinogens, dissociative drugs, synthetic opioids, and benzodiazepines (e.g., Dargan and Wood 2013; EMCDDA 2015; Baumann et al. 2017; UNODC 2018; Evans-Brown and Sedefov 2018).

Since information about dose and effect is typically not available, the concept of self-experimentation and of how these experiments are structured introduces an expanded meaning relative to the classification introduced above. For example, they might serve as a "first evaluation" of a substance's unknown effects, its

"most effective" route of administration, and its potential dangers. In order to exchange information about their experiences (and read about those reported by others), substance users refer themselves to specific websites, which are organized in a forum format that allows users to post (frequently unedited) information to help exchanging information on the drugs in question. Normally, there is no explicit design for the experiment, no formal professional education in scientific observation and methodology, and no knowledge of identity and purity of the substances involved. In this context, experimentation might therefore be considered as part of a broadened version of "wild" SEs of the third category (see above).

In respect to SEs with NPS, some additional issues might have to be considered:

1. The time span covering the use of NPS (e.g., 2005–2018) is relatively small compared to other psychoactive substances.
2. The clinical and experimental environment of today does not easily allow for SEs with NPS due to the specific requirements placed on clinical studies (availability of nonclinical toxicological data, good clinical practice, etc.).
3. In countries where implementing self-experimentation with NPS may be more difficult due to existing drug control legislation, people who opt for ingesting NPS cannot normally publish their results in the scientific domain but might place their (non-standardized) "trip reports" in Internet forums.
4. Virtually no detailed SEs with NPS were published in the scientific domain.

Nevertheless, as far as the evaluation of Internet forum contributions is concerned, information about effects of some NPS has been extracted and published using qualitative analysis tools (e.g., Kjellgren and Soussan 2011; Kjellgren et al. 2013; Kjellgren and Jonsson 2013; Soussan and Kjellgren 2014, 2015; Van Hout 2014; Erowid and Erowid 2015; Swogger et al. 2015; Van Hout and Hearne 2015a, b; Hearne and Van Hout 2016; Assi et al. 2017; Abouchedid et al. 2018).

As far as SEs with NPS are concerned, some notable exceptions exist where controlled self-experiments (category 1) have provided valuable information. For example, this was demonstrated for first-generation synthetic cannabinoid receptor agonists (SCRAs) when confirming the psychoactive nature of ingredients suspected to be present in branded "legal highs" (Auwärter et al. 2009). Interestingly, oral administration of the SCRA AM-2201 showed that the compound was not psychoactive at the dose tested (5 mg) and that the metabolic transformation resulted in the formation of some compounds that were also observed to be metabolites detected in closely related SCRAs (Hutter et al. 2013). A variety of challenges arises within a clinical and toxicological context. For example, initial drug-screening procedures based on immunochemical assays are normally not able to identify a specific NPS although sufficient cross-reactivity might exist to enable the identification of a potential drug or drug class. Other analytical difficulties might include the need for targeting the metabolites instead of the parent drug, most notably particular sample matrices (e.g., urine); thus, having data available on the metabolic fate and pharmacokinetic parameters strengthens the ability to identify a newly and previously unknown NPS in biological sample material (Wagmann and Maurer 2018;

Meyer 2018). In addition, it has been frequently noted that the biotransformation of some drugs can result in the formation of metabolites that are both pharmacologically active and which are also medicines in their own right. One of the examples where this has been identified could be found with a number of NPS-based benzodiazepines, and the fact that self-experiments have been carried out to shed light on these phenomena revealed important contributions to understanding these mechanisms. In addition, these experiments revealed significant differences in drug potency and detectability in biological samples over time (Moosmann et al. 2013a, b, 2014; Kintz et al. 2017; Huppertz et al. 2018; Ameline et al. 2018).

However, the consideration of non-standardized experiments resulting in non-standardized descriptions of drug experiences ("trip reports") and discussions on these online forums (e.g., erowid.org, bluelight.org, drugs-forum.com, or reddit.com) is beyond the scope of this chapter. Peer-to-peer generated knowledge and a social support system in respect to knowledge exchange and harm reduction can be important pillars of such a drug discourse, but it is hard to extract and evaluate the quality of those reports from these uncontrolled or "wild" experiments. As the above-mentioned publications have shown, these non-standardized reports from non-standardized experiments can just be taken as anecdotal evidence. Their use in the scientific domain is at best limited to extractions from many reports to gain a rough "mean" impression regarding their usual pattern of effects. At the same time, it is unclear whether these forums also inspire drug taking by providing these descriptions. Many users actively contributing to those forums, including descriptions of their SEs, appear to be experienced drug users driven by a desire for recreation, pleasure, novelty, and a range of functional or compensatory purposes fulfilled with their substance use.

A recent online survey found that motivations for this self-selected sample of NPS users have to be reportedly based on safer and more convenient drug use, satisfaction of curiosity and interest in drug effects, fulfillment of a sense of adventure, promotion of self-exploration and personal growth, functioning as coping mechanism, performance enhancement, facilitation of social bonding and belonging, and a means for recreation and pleasure (Soussan et al. 2018). Obviously, the motivations of users typically differ depending on the substance of choice. Whilst users of hallucinogens and entactogens appear to be mainly oriented toward self-exploration and occasional use pattern, many users of synthetic opioids are seemingly trying to cope with symptoms and clinical features associated with psychiatric disorders including opioid dependence.

6 Self-Experimentation with Various Other Psychoactive Substances

In the following subchapters, an overview on SEs of other psychoactive substances used will be provided. As mentioned before, this review is limited to hallucinogens, entactogens, cannabis, cocaine, and some dissociative hallucinogens.

6.1 Nitrous Oxide

Nitrous oxide or laughing gas was discovered in 1772 by the British inventor Joseph Priestley (1733–1804). In 1799, Thomas Beddoes, a British physician from Bristol, opened a small experimental clinic and laboratory, where he experimented on the therapeutic use of different gases, including nitrous oxide. Based on his first SEs, he got the impression that "... there seems to be quick and wrong alterations in the degree of illumination of all surrounding objects; and I felt as if composed of fine strings ..." (quoted in Shedlin et al. [1973] 1992, p. 11). It was Beddoes who hired Humphrey Davy, a self-educated student of medicine, as his assistant and gave him equipment and encouragement to pursue further experiments (Davy 1800). Davy believed in self-experimentation, which proved to be a pleasurable activity as far as nitrous oxide was concerned, though very difficult to relate and express in scientific terms. After experimenting on himself between 1799 and 1800, he wrote on the subjective experiences. An assistant of Beddoes reported on the challenges faced with the research on these fleeting experiences: "...the nature of the sensations themselves which bore greater resemblance to a half delirious dream than to any distinct state of mind capable of being accurately remembered, contributes ... to increase the difficulty" (quoted from Shedlin et al. [1973] 1992, p. 13).

After having a hard time of producing pure nitrous oxide, Davy saw no other options than self-experimenting with the gas because it was felt that animal experimentation was not considered workable. With a careful scientific attitude, he reports: "I was aware of the dangers of this experiment. ... I thought that the effects might be possibly depressing and painful, but there were many reasons ... to believe that a single inspiration of a gas ... could neither destroy nor immediately injure the powers of life" (Davy in Shedlin et al. [1973] 1992, p. 55). After using very low doses at first, further experiments were undertaken using higher dosage and extended length of administration but in the presence of a physician. When Davy increased the length of administration, "... vivid ideas passed rapidly through the mind, and voluntary power was altogether destroyed." However, he felt an immediate "desire of increasing the pleasurable feelings. ... Sometimes I manifested my pleasure by stamping or laughing only, at the times by dancing around the room and vociferating. ... Sometimes I had the feeling of intense intoxication, attended with but little pleasure; at other times, sublime emotions connected with vivid ideas" (Davy, quoted in Shedlin et al. [1973] 1992, p. 14). Davy first described the pain-relieving properties of nitrous oxide in his book although its potential use as an anesthetic was not discovered until 40 years later.

Following experiments with anesthetics other than nitrous oxide performed over a period of 14 years, philosopher Benjamin P. Blood (1832–1919) claimed that he gained revelatory insights "in which the genius of being is revealed; but it cannot remembered in the normal condition ... there is a comfort of serenity and ancient peace; while for the resolved and imperious spirit there are majesty and supremacy unspeakable" (Blood 1874, quoted in Shedlin et al. [1973] 1992, pp. 73–74). Blood concluded from his research that "the lesson is one of central safety: the Kingdom is within us. All days are judgment days; but there can be no climacteric purpose of

eternity, nor any scheme of the whole. The astronomer abridges the row of bewildering figures by increasing his unit of measurement: so we may reuse the distracting multiplicity of things to the unity for which each of us stands" (Blood 1874, quoted in Shedlin et al. [1973] 1992, p. 76).

The prominent American psychologist, physician, and philosopher William James (1842–1910) came across the writings of Blood and was eager to conduct his own SEs with nitrous oxide (James 1882). In one of his seminal publications entitled *The Varieties of Religious Experience* (James 1902), he described its effects as "revelations of significant metaphysical insights" but found himself unable to remember the exact contents of the experience. Nevertheless, he strongly urged others "to repeat the experiment to gather experiences with this extraordinary state of consciousness." According to James, ". . . the keynote of the experience is the tremendously exciting sense of an intense metaphysical illumination. Truth lies open to the view in depth beneath depth of almost blinding evidence. The mind sees all the logical relations of being with an apparent subtlety and instantaneity to which usual consciousness offers no parallel . . .." However, his enthusiasm seemed limited: ". . . as sobriety returns, the feelings of insight fades, and one is left staring vacantly at a few disjointed words and phrases, as one stares at a cadaverous-looking snow peak from which the sunset glow has just fled . . ." (James, quoted in Shedlin et al. [1973] 1992, p. 77). Following extensive numbers of SEs, James was frustrated with any attempt to measure the experience but concluded ". . . that our normal, waking consciousness, rational consciousness as we call it, is but one special type of consciousness, whilst all about it, parted from it by the flimsiest of screens, there lie potential forms of consciousness entirely different. . . . no account of the universe in its totality can be final which leaves these other forms of consciousness quite disregarded" (James 1902). According to historian Mike Jay (2009), nitrous oxide emerged as the first synthetic psychoactive substance that triggered systematic research involving SEs on the nature of the subjective experience.

6.2 Cannabis

It is impossible to nail down when the first SEs took place with this most prominent psychoactive drug that is the cannabis plant (*Cannabis sativa*, etc.) forming the resin hashish. An early nonmedical self-experimenter with this drug was Fitzhugh Ludlow, who ingested large doses of cannabis resin and gave eloquent descriptions of their subjective effects. He also noted correctly the relation between dose and effect, inter- and intraindividual variations in response, and the influence of set and setting. His autobiographical book *The Hasheesh Eater* (Ludlow 1857) created popular interest in hashish in the USA, leading to private hashish clubs. Ludlow also recorded the development of dependence and the subsequent struggle experienced with breaking the habit (Dulchinos 1999).

The studies on cannabis inebriation carried out in the mid-1920s by the German physicians Ernst Joël and Fritz Fränkel were predominantly based on their SEs (Joël and Fränkel 1926). The authors criticized the pharmacopsychological research of

psychiatrist Emil Kraepelin, which were felt to just register isolated measures. Joël and Fränkel were ambitious to contrast this approach with their "method of experimental psychopathology", which looked for influences of psychoactive substances on the "whole person" and their performance. Their SEs were intended to be "an experimental probe into the anomalous life of the soul." Following some initial animal experiments, their SEs revealed a state of intoxication characterized by a steady change between a dreamy and nearly usual waking state. Mood and affects were changing and ranged from feelings of perplexity, fragmentation, feelings of wishless euphoria, or ecstatic rapture. Trains of thought were altered, sometimes enriched by additional associations, sometimes disturbed or interrupted. Memory was found to be dysfunctional. Joël and Fränkel pointed to the "didactic" significance of SEs when used to "produce and observe artificial mental illnesses. Ideally, these drug-effects have to be short-lasting and be free from grave somatic side-effects as well as lasting after-effects" (Fränkel and Joël 1927, p. 83). Colleagues of Joël and Fränkel at the psychiatric clinic in Munich were also conducting SEs with cannabis extracts in the mid-1920s, but did not publish many details (Kant and Kropf 1928). In 1930, psychiatrist Kurt Beringer and some colleagues also conducted SEs with cannabis (Beringer 1932).

6.3 Cocaine

The first scientist to report on SEs with cocaine was the Italian anthropologist, physiologist, and neurologist Paolo Mantegazza (1831–1910). His response to the psychological effects was enthusiastic. "Little by little, one starts to feel that the nervous powers are increasing; life is becoming more active and intense; and one feels stronger, more agile, and readier for any kind of work" (Mantegazza [1859] 1973, p. 38). When he increased the dose, he felt "being isolated from the external world. One also feels deeply joyful and intensely alive." He also increased the dose to the maximum and ". . . experienced the delirium of coca intoxication, and I must confess that I found this pleasure by far superior to all other physical sensations previously known to me. . . . I sneered at the poor mortals condemned to live in this valley of tears while I, carried on the wings of two leaves of coca, went flying through the spaces of 77,438 worlds, each more splendid than the one before" (Mantegazza ([1859] 1973). However, no serious aftereffects resulted from his experimentation.

In 1884, Sigmund Freud, the creator of psychoanalysis, famously conducted SEs with cocaine over a period of years. At first, he ordered several grams of this drug to study its physiological effects after having read about its use by American Indians. In a first SE, 50 mg of cocaine eliminated his bad mood for a day, without decreasing physical or psychical energy (Freud 1884). Coming from this positive experience, he extended his use to treat his well-known melancholia. He enthusiastically recommended cocaine to others (Freud 1885a). Based on his SEs, Freud described "cheered up and persistent euphoria that cannot be differentiated from a normal euphoria observed in healthy people . . . One feels an increase in self-control, more

vigor and more able to work …" (Freud 1884). Freud also lectured about his experiences and his intention to use it on a broader scale (Freud 1885b). At this point in time, cocaine was not known as a recreational drug, and the problem of dependence was not on Freud's mind. Freud concluded that cocaine could be easily applied in cases of "neurasthenia" and melancholia. However, just 2 years later, Freud's euphoria was over when he discovered cocaine's dependence potential.

The American physician Ring (1887) wrote on "Cocaine and its fascinations, from a personal experience" for the purpose of evaluating its risk potential. Originally, he used cocaine for chronic pharyngitis but began to enjoy its euphoric effects and became "dangerously attached to the drug."

Aleister Crowley (1875–1947) was a former medical student and British magician engaged in the use of science to establish more objective methods for magic and for reaching certain states of consciousness. Since the 1910s, Crowley used hashish and cocaine on a regular basis and sometimes mescaline. Cocaine was his favorite drug, as evidenced by his flowery description of its exhilarating effects. Crowley's diaries show that he experienced the full spectrum of cocaine's effects, including unpleasant hallucinations, paranoia, and dependence, which can turn its user into a "slave of cocaine." On the other hand, he pointed to artists as examples for its productive and creative use. However, later in his life, he lost control over his use of the drug (Crowley [1917] 1973).

In the late 1920s, Ernst Joël and Fritz Fränkel also published a significant monograph on its effects and dependence-producing properties (Joël and Fränkel 1924). Their detailed descriptions show an intimate knowledge about the effects of the drug, suggesting that their writings also profited from SEs with the drug. The work based on SEs as well as experiments with artists revealed an elevation of mood and an increase in self-confidence. The intellectual abilities seemed to be subjectively increased although this did rarely led to what is considered as "lasting creations" (Joël and Fränkel 1924, p. 1031). Later SEs included combinations of cannabis with cocaine, and it was found that the effects of cannabis were significantly decreased if not completely eliminated by cocaine (Joël and Fränkel 1929). Both physicians were very aware of the dependence-producing potential of the drug and fought against the black market and illegal distribution. Lewin placed a serious warning at the end of his book chapter on cocaine: "During recent years I have seen among men of science frightful symptoms to the craving for cocaine. Those who believe they can enter the temple of happiness through this gate of pleasure purchase their momentary delights at the cost of body and soul. They speedily pass through the gate of unhappiness into the night of the abyss" (Lewin 1998, p. 74).

6.4 Hallucinogens

6.4.1 Mescaline

In 1896, and following some initial SEs reported by Prentiss and Morgan (1895), the prominent American neurologist Weir Mitchell (1896) performed a SE with two and a half buttons of the mescaline-containing peyote cactus (*Lophophora williamsii*).

He experienced an endless display of richly finished Gothic towers, statues, spinning hoops laden with jewels, and other marvels when he closed his eyes. Interestingly, his critical faculties remained intact during the intoxication phase, when he had the "... decisive impression that I was more competent in mind than in my everyday moods. ... the sense of increased ability was so notable that, liking to test it ... I took up a certain paper on psychology, which a week before I had laid down in despair. I grieve to say that it was less to be comprehended than ever. My ignorance would have remained bliss had I not made the experiment" (Weir Mitchell 1896, p. 1626). Weir Mitchell's report inspired Havelock Ellis, a prominent British physician, to carry out a SE with mescaline a year later where he ingested a decoction made from three buttons. His descriptions highlighted significant changes in his visual perception and concluded that "...ever since this experience I have been more aesthetically sensitive than I was before to the more delicate phenomena of light and shade and color" (Ellis 1898, p. 134).

Louis Lewin, professor of pharmacology in Germany, and the first systematic explorer of psychoactive substances (Lewin 1924), and Arthur Heffter (Fig. 2), a leading German pharmacologist (and founder of the Handbook of Experimental Pharmacology), were the first researchers who self-experimented with extracts from the mescaline-containing peyote cactus. Experiments on animals were conducted in the first instance in order to learn about physiological reactions and toxicity (Heffter 1894; Lewin 1888). In a series of six elf-experiments, Heffter self-administered different fractions of his plant extracts to evaluate the fraction containing the main active principle (Heffter 1898). Nearly a hundred years later, an institute established for advancing the research on psychedelic substances was named The Heffter Research Institute (www.heffter.org).

Fig. 2 German pharmacologist Arthur Heffter (1859–1925) and founder of the Handbook of Experimental Pharmacology. Courtesy of the Archives of Humboldt University, Berlin (Germany)

The 1920s witnessed many human studies with mescaline. Approximately one third of these experiments were SEs performed by physicians (Passie 2005). A few significant examples should be mentioned in some detail. In 1912, Knauer and Maloney gave mescaline (0.15–0.2 g, im) to nine physicians to compare inter- and intraindividual drug-induced effects. The authors mention that "We may see the whole symptomatology clearer if psychiatrists themselves could live through the experience of psychosis. Since this experience is usually not accessible to us, the only way to induces transitory psychosis is through the intake of such substances" (Knauer and Maloney 1913, p. 426). Guttmann (1921) refers to SEs with mescaline conducted by himself and some of his colleagues. Their aim was to get a grip on "abnormal psychological processes" by observing them in SEs. Guttmann reported cognitive and mental irritation but also elation of mood and transcendence of time and space. He also drew parallels to dreams and hypnagogic states.

In the early 1920s, the prominent German psychiatrist Kurt Beringer commenced his research on the "mescaline inebriation." Before he began to experiment, he took part in a study that tested the influence of mescaline on arithmetic, speech, and memory performance. It appears that physicians at the department volunteered for these experiments (Alberts 1921). Beringer's intention was through the ". . . experimental generation of misperceptions to analyze them more closely through introspection and the changing nature of the experimental conditions" (Beringer 1923, p. 426). Beringer's groundbreaking monograph on the "mescaline inebriation" provided a systematic evaluation of the psychopathological phenomena produced, including 50 self-descriptions obtained from his volunteers (Beringer 1927). Some of his subjects reported exhilarating nirvana-like experiences, which remained significant to them for a long time afterward (e.g., Prinzhorn 1927, 1928). During the 1950s, Beringer's follower, professor Hans Ruffin at Freiburg University, gave assistant doctors a shot of mescaline for a SEs after which they took part in the usual routines on the wards. The volunteers became sometimes quite irritated, and in this case, it was part of the experiment that other (already mescaline-experienced) doctors tried to "talk them down" (Passie 2005).

During the 1920s, the German psychiatrist Konrad Zucker conducted experimental research using different psychoactive substances, including cannabis and mescaline. He considered SEs a requirement for understanding the main features of the state of intoxication and to conduct experiments appropriately (Zucker 1926).

In the early 1930s, the physician and psychologist Hans Friedrichs at Bonn University in Germany conducted experiments in which he collaborated with psychologists who were able to describe the complex experiences induced by mescaline in detail. Friedrichs' experiments included a special feature that made his experiments unique in the early history of research with hallucinogens: "A strict standardization of external experimental conditions was to a large extent abandoned. Through maximal adjustment of the external situation and freedom the individual character of the inebriation should unfold in its own original form" (Friedrichs [1940] 2009, p. 7). His recently rediscovered dissertation on these experiments

represents the most detailed psychological study on the mescaline intoxication up to now (Friedrichs [1940] 2009).

In 1925, the German-born American neurologist Heinrich Klüver ingested some peyote buttons in his laboratory at the University of Minnesota (Klüver 1966). According to himself, he performed this SE "... not for the sake of consciousness expansion or other unique experiences, but to test a new tool possibly useful in studying various problems of the psychology and pathology of perception [and] ... the study of certain types of pseudohallucinations ..." (Klüver 1980, p. VIII).

Psychopharmacologist Roland Fischer began his long career of hallucinogen research with a SE with mescaline in 1945, which was published in the form of a detailed account (Fischer 1946). The professor of psychiatry, Norbert Matussek (1952), at the Max Planck Institute of Psychiatry in Munich (Germany) also conducted some SEs with mescaline and two other physicians to gain insights into the nature of psychotic states. The Canadian psychiatrist Humphrey Osmond was also not shy of performing SEs and took also part in a Native American ceremony in 1956 centered around the ingestion of peyote (Osmond 1970). Similarly, this was also done by two other prominent psychiatrists (Ammon and Patterson 1971).

6.4.2 Lysergic Acid Diethylamide (LSD)

The psychological effects of LSD were discovered in 1943 by Albert Hofmann in the laboratories of the pharmaceutical company Sandoz in Basle (Switzerland). At first, he got accidentally intoxicated in his laboratory and felt some strange sensations. Shortly thereafter, he conducted a SE with LSD and found it to be active in extremely small quantities as low as 0.1 mg. Further SEs by his laboratory co-workers followed soon (Hofmann 1955). Somewhat later, a whole series of SEs was performed by Solms to evaluate the activity of some derivatives of LSD (Solms 1956). It is obvious from the publication on the first clinical studies on LSD that most volunteers were physicians located at the Psychiatric Clinic at the University of Zürich (Switzerland) (Stoll 1947; Condrau 1949). Interestingly, virtually all studies conducted with LSD until the mid-1950s were SEs and/or employed physicians as subjects (e.g., Becker 1949; Mayer-Gross et al. 1951; Weyl 1951; Arnold and Hoff 1953; Frederking 1955).

The psychoactive effects of LSD were discovered and inspired significant research efforts across scientific disciplines, especially in psychiatry. For example, the Canadian psychiatrist Humphry Osmond (who later coined the term "psychedelic" = mind-manifesting) was curious about gaining insights into the subjective world of the schizophrenic mind by taking a variety of hallucinogens (cf. Hoffer and Osmond 1967). In the mid-1950s, Osmond let the internationally prominent writer Aldous Huxley take mescaline under his supervision. Following this experience, Huxley became a major spokesman for the intelligent use of psychedelics (Huxley 1954, 1980).

In the USA, the first researchers who came in touch with LSD in the early 1950s were Max Rinkel and Sidney Cohen who were not shy to experiment on themselves with the new drug. Cohen took the drug in October 1955 expecting to feel catatonic, paranoid, or confused but found himself "... taken by surprise. This was no

confused, disoriented delirium, but something quite different." He described feeling an elevated peacefulness, as if "the problems and strivings, the worries and frustrations of everyday life vanished; in their place was a majestic, sunlit, heavenly inner quietude . . . I seemed to have finally arrived at the contemplation of the eternal truth" (Cohen 1964, p. 107). After having finished three dissertations on psychological testing of subjects under LSD, he wrote: "Though we have been using the available measuring instruments, the check lists, the performance tests, the psychological batteries, and so forth, the core of the LSD situation remains in the dark, quite untouched by our activities" (Cohen 1967, p. 11). Hoping for more articulate reports, Cohen turned to his friend Gerald Heard, a freelance writer in mysticism and popular science. Heard described LSD's effects as "a shift in consciousness" that was "so clearly similar to the accounts given by the mystics that none of us feel able to deny that this is in fact the experience which we undergo" (Heard, quoted in Novak 1997, p. 93).

Another significant figure in early LSD research in therapy and creativity was the Californian psychiatrist Oscar Janiger. Following Janiger's first SE with LSD in 1954, and more than 10 thereafter, he set up a "naturalistic study" involving 875 people who had been introduced to LSD with many of them being part of the creative community in Beverly Hills and Hollywood, including Anais Nin, Cary Grant, and Jack Nicholson (Stafford 1990).

In the early 1950s, experimentation with LSD began at the Psychiatric Research Institute in Prague (Czechoslovakia). One of the first subjects to self-administer the drug was the internationally known psychiatrist and LSD therapist Stanislav Grof. Sitting in front of a strong flicker light during the initial phase of the experiment, he was catapulted through eons of time and space and felt his consciousness expanded beyond all boundaries. After the Prague spring in 1967, he left for the USA and led the last research center for the therapeutic use of psychedelic drugs in Baltimore, Maryland, until 1976. At this center, psychiatrists and nursing personnel were involved in SEs aiming to learn both about their patients' psychotic crises and also therapeutic processes (Grof 1980).

In 1959, LSD was at its peak of medical acceptance although Cohen detected trends of going lax in controlling the drug and its use. Researchers immersed in SEs began to share LSD in their homes and introduced others to the experience. In 1958, it was reported that researchers held "LSD-25 social parties" and that LSD became "an intellectual fun drug" (Ditman, quoted in Novak 1997, p. 99). In the late 1950s, prominent (and CIA-associated) LSD researcher Harold Abramson held Friday-night soirees in his home and was "besieged by people who wanted to take the drug" (Abramson 1967, pp. 33, 475; cf. Novak 1997, p. 99). In 1960, Cohen felt very much "uncomfortably unscientific" and wrote to his sponsor that he got enough "of the fringy goings on with this group of drugs." The Federal Drug Administration's first investigations on the abuse of LSD began in 1961 in Southern California, where "reports of misuse" focused on "physicians and psychologists who were not authorized to use the drug" (Novak 1997, p. 108). In 1962, the police raided several therapists using LSD in the Los Angeles area and seized their LSD supply. However, a well-informed psychiatrist working for the US Army stated: "in the early 1960s,

practically every LSD investigator in the nation had taken LSD at least once, if only to become familiar with the subjective effects. Many, of course, took it innumerable times, incorporating it into their life style and self-concept" (Ketchum 2006, pp. 67–68). Ketchum also performed a SE with 80 μg of LSD in 1965, but without reporting significant insights (Ketchum 2006).

In 1961, some physicians and pharmacologists at the Psychiatric University Clinic in Zürich conducted systematic SEs to compare the effects of LSD, psilocybin, and ethanol. The experiments were recorded on tape and used later in lectures about the effects of drugs (Waser 1990).

Since the late 1950s, psychologist van Dusen (1961) conducted SEs with LSD at the Mendocino State Hospital in Talmage, California (USA). He concluded "there is a central human experience which alters all other experiences. . . . I wish to draw attention to the fact that the still experimental drug . . . (LSD) appears to facilitate the discovery of this apparently ancient and universal experience" (van Dusen 1961, p. 11).

A group around professor of psychology Timothy Leary commenced research into the psychological effects of psychedelics after they had conducted SEs with psilocybin in 1961. They administered psilocybin in a "supportive environment" to volunteers. In some experiments, an experimenter took the drug together with the experimental subjects (Leary et al. 1963). A short while later, their ambitions to propagandize "consciousness-expansion" became so impertinent that they left Harvard University for conducting social experiments. The group opened a "psychedelic center" in Zihuatanejo (Mexico), where they explored regular psychedelic drug use and experimental social ways of life (Downing 1965). Thirty-five people, mostly psychologists, studied "the transpersonative effects of group interaction with the concurrent use of LSD." The group promoted the view that "stereotyped learned patterns, or 'games', created by familial and social pressures . . . are considered to inhibit direct person-to-person contact" (Downing 1965, p. 150). After leaving Mexico, the group opened a center for "psychedelic exploration" in Upper New York. There the group continued working on SEs and began to proselytize, to "turn on the world," feeling that the psychedelic experience furthers a new consciousness which would be leading to a "new age" (Hollingshead 1973). With their "wow"-approach, the group soon attracted the attention of the media, the world, and finally the police. Partially as a result of this, most hallucinogenic substances became controlled in 1966 in the USA.

In the 1950s, physician and neuroscientist John C. Lilly began experimenting with the "isolation tank," in which a person is completely isolated from any sensory perception while being immersed in a salt water solution heated at body temperature. After getting accustomed to these special circumstances, Lilly conducted SEs with LSD in the tank. The resulting books later became classics in "consciousness expansion" and the "spiritual search" literature of the 1970s (e.g., Lilly 1972a, b, 1978).

Dozens of Czech psychology students underwent LSD experiments under the supervision of psychiatrist Stanislav Kratochvíl's team at the psychiatric hospital at Kromeriz. The group's approach focused on didactic and autognostic sessions.

Kratochvíl and his team believed that there is "a significant purpose of the didactic experiments for understanding some mental states occurring during psychosis; for enabling the study of psychopathology at a graduate and postgraduate level; for expanding the understanding of oneself; and for personal growth" (Kratochvíl S, Užití LSD v psychiatrické léčebně v Kroměříži v roce 1966 [The use of LSD in the psychiatric hospital in Kroměříž in 1966]. Unpublished document, 1967, p. 1).

It has to be mentioned that all physicians who founded the Swiss Physicians Society for Psycholytic Therapy (SÄPT) in 1986, which still exists as a knowledgeable institution today, carried out many SEs with LSD, mescaline, psilocybin, and MDMA (Benz 1989).

6.4.3 Nightshade Hallucinogens

When it comes to the somewhat rarely used traditional plant hallucinogens of the nightshade family (*Datura* spp., *Atropa belladonna*, *Hyoscyamus niger*, etc.), which contain scopolamine and hyoscyamine (easily converted to atropine) with hallucinogenic properties, SEs have been rarely reported. That might be due to the fact that the intoxication provoked by these plants induces rather unpleasant physiological and psychological effects. Konrad (1888) and Klinke (1889) conducted the first scientifically driven SEs with scopolamine to explore the effects. Prominent psychiatrist Oswald Bumke (1903) at the University of Munich (Germany) conducted SEs with low doses of scopolamine. The studies of research psychiatrist Hans Heimann, which led to the only monograph on effects of scopolamine, were based on SEs he had conducted to design his study (Heimann 1952). Another focus about the nightshade plants were the "witches ointments" (Hexensalben), which have been used by medieval witches to "travel to the sabbath". In the early 1950s, German ethnologist Will-Erich Peuckert prepared such an ointment following a recipe provided by Giambattista della Porta and smeared it on parts of his body. He recounted afterwards: "We had wild dreams. Horrifically distorted faces danced in front of my eyes. I then suddenly had the feeling of flying through the air. The flight was interrupted repeatedly by massive plunges. In the final phase . . . the image of an orgiastic celebration with grotesque sensual excesses" (Peuckert cited in Wellen 1986, p. 158). Similar experiences have been reported by another self-experimenter using such a preparation (Ferkel 1954).

6.4.4 Fly Agaric (*Amanita muscaria*)

In 1967, Swiss pharmacologist Peter G. Waser and psychiatrist Jules Angst performed SEs with some compounds isolated from the fly agaric mushroom (*Amanita muscaria*). Muscimol and ibotenic acid reportedly produced hallucinations, disturbances of consciousness, as well as time and space perception. Especially remarkable was the perception of "gliding through infinite spaces like on ice and to repeatedly re-experience the situations and sounds in reverberating images." These investigations confirmed the experiences reported from Siberian shamans and their use of this mushroom (Waser 1990, p. 57).

6.4.5 *Salvia divinorum*

The hallucinogenic effects of the Mexican sage *Salvia divinorum* were first reported by the Swedish anthropologist Jean Bassett Johnson in 1938. In the late 1950s, ethnomycologist Robert G. Wasson and LSD discoverer Albert Hofmann took part in a shamanic ceremony by chewing the leaves. They reported mild hallucinogenic effects (Hofmann 1979). Quite a while later (in 1979), researchers took part in another ceremony where a higher dose was given that resulted in more pronounced hallucinogenic effects. In 1982, the active principles (salvinorin A and B) were isolated. Since then, administering higher doses became a possibility, and researchers began to use highly concentrated extracts in SEs to discover powerful hallucinogenic effects and alienating dissociative states. Through SEs, researcher Daniel Siebert found that salvinorin A was not orally active but that it required absorption through the mouth mucosa (Siebert 1994). It is also very effective when smoked. According to reports, salvinorin A effects enter with an irresistibly powerful force that takes the user in a dissociative trance state. After some initial body effects, the user is catapulted into strange realms of experiences. Sometimes, experiences might involve the most cosmic, wonderful, and detailed universes, while at other times, memories might not be recalled. A drastic shift in sense of identity and conscious perception has been reported, usually completely dislodged from the usual body experience and the familiar sense of self or ego. Sometimes, it appears that one has ceased to exist as a body, human, or soul. Some feel a sensation that their "being" can literally enter and inhabit various objects (Siebert 1994; Turner 1996). It appears that virtually all significant researchers of *Salvia divinorum* and its active principles have engaged in self-experimentation (Wasson 1962; Valdes et al. 1982; Siebert 1994; Turner 1996; Arthur 2008). It is noteworthy that this plant/substance does not show any dependence potential, and many users appear to stop its use when confronted with an unpleasant experience (Gonzalez et al. 2006; Maqueda et al. 2015).

6.4.6 *N,N*-Dimethyltryptamine (DMT)

Not much can be said about SEs performed with DMT and closely related substances. Some marginal self-experimentation was going on with these substances during the 1950s and 1960s. Stephen Szára and colleagues (see below) relied on SEs to explore the "psychopathological" effects of DMT and some of its derivatives, which were suspected to be linked to "pathological metabolites" and psychotic states.

Szára took mescaline in 1955, and when he was unable to obtain LSD, he turned to DMT for further experiments. After experiments with cats, he discovered that DMT was inactive when given orally, thus deciding to administer the substance intramuscularly. He described that "The hallucinations consisted of moving, brilliantly colored oriental motifs, and later I saw wonderful scenes altering very rapidly. The faces of the people seemed to be masks. My emotional state was elevated sometimes up to euphoria … My consciousness was completely filled by hallucinations, and my attention was firmly bound to them" (Szára 1957, p. 462). The researchers experimented with different modes of administration and were able to describe a comprehensive clinical picture of DMT's psychological effects (Szára

1957). It became obvious through these SEs that the intensity of effects was significantly linked to the route of administration with nasal insufflation and smoking leading to the most drastic experiences. Later on, Szára also tested closely related hallucinogenic tryptamine derivatives on himself. According to Szára, the effects of these drugs supported "the aminotoxic and indole theory of schizophrenia" (Szára 1961).

One might also mention the SEs with DMT carried out by Timothy Leary. Leary got some intramuscular injections of DMT in 1965: "Suddenly I opened my eyes and sat up . . . the room was celestial, glowing with radiant illumination . . . light, light, light . . . the people present were transfigured . . . god-like creatures . . . we were all united as one organism. Beneath the radiant surface I could see the delicate, wondrous body machinery of each person, the network of muscle and vein and bone – exquisitely beautiful and all joined, all part of the same process" (Leary 1966, p. 86).

As it appears from these SEs, the state of consciousness experienced during the initial intoxication is characterized by amazing visual effects. However, this seemed to be just a prelude to a profound state in which subjects report contacts with "another realm of reality" in which they might encounter discarnate, nonhuman alien beings. "I passed abruptly through to another realm, losing all awareness of my body. It was as if there were alien beings there waiting for me, and . . . spoke to me as if they had been awaiting my arrival. . . . the entities approached me from the front, rapidly and repeatedly, appearing to enter and pass through me" (Meyer 1993, p. 43).

Administrations via intravenous, inhalation, or nasal routes invariably lead to experiences usually so bizarre and dramatic that an inexperienced person might feel like being catapulted out of any known realm of consciousness. Referring to the impression of encountering "discarnate entities in another realm of reality," Peter Meyer elaborated on these encounters following his SEs and those of others (Meyer 1993). A similar direction was pointed toward the research of ethnobotanist and anthropologist Terence McKenna, who experimented with LSD, psilocybin, and DMT. McKenna became a prominent spokesman of the "psychedelic movement" during the 1990s and thereby facilitated the research and distribution of psilocybin (Oss and Oeric 1976) and DMT (McKenna 1991).

More controlled and comprehensive SEs were reported by the ethnopharmacologist Jonathan Ott. He also explored the possibilities of producing an orally active DMT-containing inebriant. Ott's research focused on possible plant mixtures other than those used in the Amazon basin associated with ayahuasca, commonly represented by specific DMT-containing plants (usually *Psychotria viridis*) combined with plant-based monoamine oxidase A inhibitors (normally *Banisteriopsis caapi*) that would render DMT orally active (Ott 1994). Ott carried out hundreds of SEs that he called "subjective bioassays." He later continued his research to explore the psychoactive effect of bufotenine (5-HO-DMT). Ott evaluated different routes of administration and found that some were more effective than others (Ott 1994, 2001a). Even though Ott was not associated with any specific university environment, his SEs followed a scientific format including influential publications in scientific journals (e.g., Ott 1999, 2001b, c).

6.4.7 Synthetic Hallucinogenic Phenethylamines

One follower of Shulgin's research can be seen in the Swiss chemist Daniel Trachsel, who published various contributions on many new psychoactive substances and their effects (Trachsel 2011, 2012; Trachsel et al. 2013). Experimental results about their psychoactive effects are included, but the author distanced himself from any SEs (Trachsel 2011, p. 12).

More ambitious explorations of subjective effects elicited by a series of new psychoactive substances developed by Shulgin were conducted by his close associate and psychologist, Myron Stolaroff. Following SEs with LSD in the mid-1950s, Stolaroff became involved in scientific research on psychedelics. After the control of most psychedelic drugs in the 1970s, Stolaroff conducted SEs with newly synthesized psychedelics 2C-B, 2C-E, 2C-T-2, 2C-T-7, 2C-T4, 2C-T-21, and MEM but also MDMA. Besides Shulgin, Stolaroff was the first who systematically explored the psychological states and their possible uses but under noncontrolled conditions (Stolaroff 1994). He understood his research as an attempt "to make the unconscious conscious" and to give some "guidelines for the proper and safe use of psychedelic drugs in therapy and for spiritual growth" (Stolaroff 1994, pp. 13–14).

6.5 Entactogens/Empathogens

When it comes to the entactogenic drugs, i.e., certain types of ring-substituted 1-phenylpropan-2-amines, it is interesting to see that this group of substances was mainly explored by chemists and pharmacologists.

The first person to experience the psychoactive effects of an entactogen was the Californian chemist and pharmacologist Gordon A. Alles. Alles discovered the psychoactive effects of amphetamine in a SE in 1925. While being interested in researching some amphetamine derivatives in 1934, he accidentally ingested a larger dose of 3,4-methylenedioxyamphetamine (MDA), which marks the first human entactogenic trip. It appears that he did not make this discovery public, because of interest that might arise from the military to be used as a potential "truth drug" useful for interrogation purposes (Passie and Benzenhöfer 2018). However, in 1959, a description of his SE appeared (Alles 1959). In the course of his secret work for the US Army, Alles synthesized other hallucinogenic/entactogenic derivatives of mescaline and probably tested them on himself.

The American chemist and pharmacologist Alexander T. Shulgin started his research on the synthesis and self-administration of psychedelic drugs after having experienced the effects of mescaline in 1960. Shulgin, most probably following Alles' research, first synthesized MDA in May 1961 for the purpose of self-administration. Since that time, Shulgin synthesized and tested (mainly on himself) hundreds of new psychoactive substances of the phenethylamine, amphetamine, and tryptamine class. After initial SEs with a newly synthesized substance, he invited some friends and fellow researchers (more than half of them scientists eager to carry out SEs) to participate in these "trials" to investigate their subjective effects (Shulgin and Shulgin 1991, 1997). In the course of these experiments, Shulgin developed a

simple rating scale in an effort to measure the intensity and the general character of the experiences (Shulgin et al. 1986). However, some scientists suggested that he biased his subjects by informing them about the general character of the substance's effects in advance. Certainly, a comparison with double-blind, randomized, placebo-controlled trials cannot be made, but Shulgin and his associates experimented in their circle for more than 25 years in a kind of systematic fashion, and many results were published in a scientific format (Shulgin and Shulgin 1991, 1997).

An interesting anecdote is that Shulgin was not able to detect the special entactogenic effects of MDE and MDMA in his (self-)experiments. Regarding MDE, his associate, the Chilean psychiatrist Claudio Naranjo, reported "no reaction" in 1967, with a low dose of MDE. Probably because of this report, no further research in the methylenedioxyamphetamine class was conducted. However, in 1975, Shulgin was contacted by a student about the idea of preparing *N*-methyl-MDA (MDMA). The product was considered "interesting" (Resnikoff 2018), but did not lead to much further testing. When Shulgin was informed by another student about the special effects of MDMA in 1976, he commenced with SEs but named its effects in his laboratory notebook as "an alcohol-like intoxication" (Benzenhöfer and Passie 2010). It was not until his friend and psychologist Leo Zeff reacted differently to a higher dose that MDMA became known to a larger circle of psychotherapists (Stolaroff 2004). However, this "failure" shows that self-experiments are subjective, provide just anecdotal evidence, and not rarely lead to wrong conclusions. Shulgin and his wife Ann let the world participate in their research by their inspirational writings (Shulgin and Shulgin 1991, 1997) and enjoyed astonishingly good health in their later years. Noteworthy is Shulgin's first synthesis, SE, and description of the subjective effects of 2C-B (Shulgin 1975).

Long before Shulgin's books were published, the physician Andrew Weil gave the first concise description of the psychoactive effects of MDA (Weil 1976) based on SEs. Weil also conducted SEs with psilocybin mushrooms and the DMT-containing "Yage" plant concoction (Weil 1980). At the same time, further work on the subjective effects of the enantiomers of MDMA and MDA, involving SEs, was published (Anderson et al. 1978). Virtually all physicians and psychologists who used MDMA in psychotherapy in the 1977–1985 period (when it was still legal) reported that SEs inspired their therapeutic work (Passie 2018).

6.6 Dissociatives (Ketamine)

In his autobiographical work "The Scientist," physician and scientist John C. Lilly described his SEs with ketamine and spread the word about its effects. Lilly suggested that ketamine enabled him to "look across the border into other realities" and to venture beyond the social consensus reality to more profound "meta-realities." Lilly also combined the use of ketamine with the flotation tank. Following experiments with electrodes and monkey brains, Lilly explained that ". . . eventually I will use myself as the subject of the experiment . . . until one is willing to undergo the experiment oneself, one must not perform them on other humans. . . . A doctor

should never give a drug to a patient until he has tried it himself" (Lilly in Kelly 1999, p. 48). However, Lilly's use of ketamine became excessive and he was temporarily diagnosed as paranoid. He believed in an "Earth Coincidence Control Office," designed by extraterrestrials to choreograph coincidences to gently push mankind down the evolutionary path.

In the late 1970s, the anesthesiologist Howard Alltounian and his wife, the yoga and astrology teacher Marcia Moore, began to explore the psychedelic effects of ketamine. During their ketamine SEs, they felt a blissful state they called *samadhi*, which subsequently led to the design of a psychospiritual treatment technique called "samadhi therapy," where they introduced these states to others for therapy and "enrichment of spiritual life." They came to believe "that in the right hands this substance could be safely, easily, and advantageously applied toward the psychospiritual regeneration of planet earth." Besides their SEs and some case histories, the authors also discussed some critical issues (Moore and Alltounian 1978). Nevertheless, a few years later, Marcia Moore disappeared and was found dead and frozen months later thought to be a consequence of an accident caused by unrealistic behavior associated with her use of ketamine.

The research by Karl Jansen, an expert on the psychedelic use of ketamine and ketamine dependence ([2001] 2004), was inspired significantly by his own SEs (Jansen and Darracot-Cankovic 2001). His scientific inquiries included photo-imaging of receptors related to ketamine experience and similarities to near-death experiences. His expertise on "ketamine addiction" was expressed in scientific articles (Jansen 2000; Jansen and Darracot-Cankovic 2001), but did not prevent him from becoming ketamine dependent himself. Therefore, the fate of Jansen, Lilly, Moore, and Alltounian point to the dangers of losing control without external control mechanisms in place in situations where self-generated SE gets "uncontrolled". This appears to be especially true when the drug has enjoyable, euphoric, or escape-promoting "dissociative" effects like ketamine. It is noteworthy that in contrast to ketamine, documented SEs carried out by scientists and descriptions of effects induced by the related drug phencyclidine (PCP or Angel Dust) and its derivatives do not appear to be available.

7 Discussion

It appears obvious that some of the first "proto-scientists" who systematically navigated the complex space linked to the use of psychoactive substances were shamans. However, nothing is really known about SEs with psychoactive drugs until the mid-eighteenth century, which triggered a long-standing tradition throughout the nineteenth and early twentieth century. However, it also appears that they became less frequent (and in most cases better controlled) after World War II.

One has to be reminded that after the turn to the twentieth century, medical research was frequently considered a "hobby" for doctors with independent incomes, and research was often seen as a luxury rather than a necessity. Prominent examples for these "private laboratory" researchers include the German pharmacologist Louis

Lewin, the American ethnomycologist Robert G. Wasson, and the American chemist Alexander Shulgin.

In this chapter, the broad range of self-experimentation with psychoactive substances since the mid-1850s is presented. These experiments began to develop slowly and on an occasional basis with the first psychoactive substances to become known in the West being cannabis and cocaine. As outlined in the present chapter, motivations, intentions, "experimental procedures," as well as the trajectories related to these SEs were quite different.

SEs require a willingness to engage in research by trial and error and to be prepared for facing potential health risks. To take this risk might become easier when certain rewards can be expected. Potential rewards might include the prospect of learning more about oneself by means of perceptions beyond the usual mental framework (seen, e.g., with psychedelic and entactogenic drugs), heightened mood, or euphoria (e.g., the euphoriant cocaine or some phenethylamine/amphetamine-based stimulants). As far as the literature published by scientists is concerned, it appears that substances with a comparatively "simple" spectrum of effects (e.g., benzodiazepines and opioids/opiates) have invited much less self-experimentation compared to drugs with more "complex" effects that impact on many spheres of the human experience (e.g., classic psychedelics) (Fig. 1).

The prospect of potentially confronting unpleasant effects such as confusion or loss of self-control (e.g., elicited by nightshade drugs atropine and hyoscyamine) presumably makes it less likely to engage in self-experimentation unless specific purposes have been identified (e.g., evaluating witches' ointments and potions). It is also obvious that experimenters did not tend to repeat them due to these unpleasant side effects. At the same time, it also seems that the classic hallucinogens radiated some form of appeal, at least to some experimenters in spite of the possibility of experiencing psychological effects that might be challenging to cope with. In comparison with other more popular substances, such as the psychostimulants that induce predictable effects, many self-experimenters might not want to be confronted with unfamiliar aspects of their personality and life experiences which, together with the somewhat incalculable course of effects, seems to restrict the use of this class of drugs perhaps to more specific user populations.

This is different with substances that regularly heighten mood, euphoria, and ego-inflation (e.g., cocaine), which have sometimes unfolded their dependence-producing properties in investigators who started the research with other intentions. Escapism ("from reality") might also play a role. A prominent example was John C. Lilly who withdrew from reality when he injected himself daily with ketamine (while lying in an isolation tank) for more than a year. However, this seems to be the exception to the general rule, which is that scientists remained in control of their self-experimentation.

7.1 Motives for Self-Experimentation

Many different motives and backgrounds can be identified when exploring the available literature on SEs with psychoactive substances. Most motives can be found in just only a very few cases and sometimes in combination, whereas others are more common. Some of the common motives include:

- Personal curiosity (Ellis)
- To explore the effects of unknown substances (Hofmann, Solms, Internet forums)
- To learn about drug effects (Beringer, Friedrichs)
- To learn how to handle the substances' effects (Hoffer and Osmond)
- To gain knowledge from the substances' effects (Davy, James)
- To search for answers to philosophical questions and inquiry (Blood, Hofmann)
- To explore new territory (Shulgin)
- To cope with psychological problems (Freud, Lilly)
- To gather power over others (Crowley, US Army)
- To learn how to manipulate others (Crowley, US Army)
- To use and risk one's own organism first (Shulgin)
- To explore possible risks (Grof, Passie)
- To optimize environments used for experiments (Passie)
- To gather information about adverse effects as harm reduction (Internet forums)
- To explore possible complications ("prepared anticipation") (Internet forums)
- Escapism (Lilly, Jansen)

Some substances serve certain purposes better than others. For example, some of the dissociative anesthetics might be more conducive to escapism, which induce a decoupling of the individual from the surrounding world (Feldman et al. 1979). In contrast, cocaine permits an "escape from reality" in respect to a more egocentric and euphoric state of mind but without profoundly altering perception of ego or reality. However, it is also probably fair to state that hardly any psychoactive substance carried such a philosophical underpinning in the way it was expressed for LSD. LSD was advocated as having a purpose other than simply "getting high". For its users, the "psychedelic experience" was about enhanced and expanded perception or "consciousness expansion." "My exponentially heightened awareness saw *through* the static, one-dimensional, ego-constricted, false front which is the consciousness-*contracted* reality of the everyday world. This was no evasive flight *from*, but a deep probe *into* reality" (Solomon 1964, p. X). LSD appeared to provide access to a numinous space unmediated by a religious hierarchy or sacred texts. Therefore, its use was predominantly experimental. A problematic pattern of repeated use was rarely, if ever, reported. In general, it appeared that substances, which "open the mind" to more emotions and unusual perceptions, were less likely to be abused because these types of substances might confront the researcher with an experience and psychological material that might be considered unpleasant and/or irritating. However, controlled and specific conditions, for example, as part of psychotherapeutic interventions, might be specifically sought after and useful.

7.2 Goals of Self-Experiments with Psychoactive Substances

From the review of the literature, a number of goals associated with self-experimental use were identifiable, and some of these were more, whereas others were less explicitly stated. The following list is meant to provide some ideas about the motives and conscious decisions made by those researchers who engaged in SEs. Some should perhaps be seen in the context of incomplete scientific knowledge and methodology:

- To identify the psychoactive constituent(s) in extracts obtained from a plant matrix
- To evaluate the general effects of the substance
- To investigate the metabolism and pharmacokinetics of a substance
- To explore risk potential
- To evaluate some specific effects of the substance
- To start a career in experimental psychopharmacology
- To explore substances with therapeutic potential
- To understand therapeutic processes of patients under the influence of the drug
- To gain personal insights into "abnormal mental states"
- To handle patients in psychotic states with more empathy
- To collect material about intoxication and to instruct students and trainees
- To explore experimental procedures from the subject's perspective
- To design appropriate experiments
- To optimize procedure and atmosphere for experiments
- To prepare for dangers potentially arising from the drug
- To evaluate new psychoactive substances for dissemination to others
- To gain philosophical insights
- To gain mystical states and insights into the human condition
- To enjoy the effects of the substances
- To enhance the drug experience
- To hold social LSD parties

7.3 Ethical Issues in Self-Experimentation with Psychoactive Substances

Not many explicit ethical statements can be found regarding self-experimentation with psychoactive substances. It appears that most of the investigations were triggered by curiosity and/or were part of larger scientific studies that included SEs (e.g., Beringer's investigations with mescaline at Heidelberg University). Systematic explorations of newly synthesized substances also provided an impetus (e.g., Shulgin).

It has to be mentioned that under the conditions operating today, SEs with psychoactive substances under controlled conditions have to be reviewed and permitted by institutional review boards (IRB) that check for compliance with ethical and scientific standards. Essential toxicological data are also required. Few

exceptions from this rule are possible depending on different laws being in force in different countries.

Ethical considerations might have played a role in SEs designed to gain insights into the condition of the mentally ill, and an important implication was to treat these patients more effectively (e.g., Ruffin at Freiburg University, Hoffer and Osmond 1967). Others were intended to develop more empathy for people experiencing psychotic states, for example, as expressed by the founder of the Soteria treatment concept applied to acute psychotic patients (Calton et al. 2008), which were inspired by their own LSD trips (Mosher 1999).

Other SEs were thought to provide insights into the treatment of patients who were treated with LSD- or psilocybin-assisted psychotherapy. Hanscarl Leuner, a "psycholytic" therapist at Göttingen University (Germany), and other like-minded psychotherapists confirmed that psychotherapists wanting to engage in hallucinogen-assisted psychotherapy had to have experience themselves in order to effectively guide patients empathically through their experiences (e.g., Passie 1997; Winkler and Csémy 2014; Grof 1980). From an ethical perspective, this has been considered as an important cornerstone of therapeutic work.

SEs with LSD were also consistent with recommendations made by the Sandoz pharmaceutical company (former Swiss producer of LSD) and were well integrated among psychiatrists and psychologists (Grof 1964). This was also congruent with the psychoanalytical tradition, in which the trainee had to go through a "teaching analysis" in which one was analyzed by an educated psychoanalyst. The purpose of this was to deepen the understanding of reaction patterns and identify "blind spots" as well as deepening the therapeutic process itself. "Auto-experimentation is a way to broaden and complement scholarly knowledge as well as to enrich and deepen a medical doctor' s understanding of those with mental illness; it is possible to say that it contributes to a more humane relationship to those with psychosis" (Roubíček 1961, p. 81).

Passie (2002) has taken part in controlled scientific experiments with psychoactive drugs prior to performing clinical studies in an effort to explore the space encountered during the drug experience. The purpose of this approach was to develop optimization strategies for the research setting and to minimize the occurrence of unpleasant experiences. The experiences resulting from such SEs informed the design of the studies and provided optimal circumstances, which are also paramount to avoiding "bad trips." This is somewhat congruent with the mode of experimentation used very early by Friedrichs ([1940] 2009), Leary et al. (1963), and McGlothlin et al. (1967). It is probably fair to assume that it is not just coincidence that all those experimenters, which provided "optimized" psychophysical environments for their subjects, had profited from SEs, which then informed their modus operandi.

7.4 Kinds and Consequences of Self-Experiments

When evaluating the three kinds of SEs identified earlier, i.e., controlled SEs, uncontrolled SEs, and "wild" SEs (Sects. 4.1.1, 4.1.2, and 4.1.3), it would appear

that the majority of the presented SEs reviewed in this chapter belonged to the first category.

Other examples however seemed to fit into the second category (e.g., Crowley, the Los Angeles group of psychotherapists, and Leary's group at Harvard). It seems that most of those researchers started with scientifically ambitious procedures first but then became successively more and more involved with the drug and its effects until the point when they withdrew from scientific conventions and turned to a somewhat "socially autistic" mode of experimentation. This obviously happened with Leary's group that "dropped out" of science and society. As far as researchers were concerned who operated on a more individual level (e.g., Crowley, Lilly), one cannot help but draw parallels with a similar kind of "autistic" syndrome.

At the same time, experimenting with certain types of psychoactive drugs can be associated with unique features and results. For example, the use of classical hallucinogens such as LSD or psilocybin, in no small part due to the often dramatic nature of the experiences induced, has been associated with changes of personality, social attitudes, and value system. In some cases, self-experimentation has led to alterations of group dynamics (e.g., Leary's group). A similar phenomenon has been observed with a leading psycholytic therapist operating in Switzerland, who founded a sect involving psychedelic substances spearheaded by him as its guru (Widmer 1997). Some observers have interpreted this as a necessary consequence derived from the experiences and insights gained from the use of psychedelic drugs, whereas others have interpreted this "drop out" behavior as a loss of control and a problematic, even dangerous behavioral change. However, this "drifting out of science" phenomenon was associated with a repeated pattern of drug use and a transition into a "wild" form of self-experimentation. However, the published data indicate that such a development was an exception rather than the rule.

7.5 Dangers of Self-Experimentation

The pursuit of self-experimentation has been repeatedly criticized for overenthusiasm, (usually) for positive bias involving data interpretation, and for lack of ability to evaluate the findings critically. As Beecher stated: "... self-experimentation is an unwise performance whenever judgement can enter into the conclusion drawn" (Beecher 1959, p. 468). The researcher involved in research is the experimental subject and the observer at the same time, especially if one aims to probe subjective psychological effects. In conventional experiments, this would be seen as a significant bias. "An enthusiastic investigator's subconscious interpretation of the results of a study in which he is an objective observer, and not a participant, could bias his study to the same degree as it would if he had included himself among the subjects" (Altman 1972, p. 351). When it comes to the study of effects other than the "subjective," for example, when performing a surgical procedure or treating an experimentally induced infection, then this might be considered a much smaller issue.

Another important point is the incalculable risk of experimenting with substances for which no basic toxicology data exist. This might be not have been as risky with substances used for SEs in the past, where for most of them, traditional human use for longer periods of time was reported (e.g., mescaline, cocaine). Especially if it comes to recently emerging NPS, no such "pretesting" exists, and the user is at high risk of overdoses, complications, and psychiatric sequelae.

A list of the possible risks associated with self-experimentation includes the following:

- Overly subjective (e.g., exaggerated) description of effects
- Lazy attitude without realizing potential dangers
- Reckless experimentation
- Loosing contact with consensus/social reality
- Unrealistic behavior
- Losing control over drug use
- Psychological complications
- Physical complications
- Overdose
- Development of dependence
- Drug taking takes center stage
- Group dynamics becomes dysfunctional
- Inspiring others to take a certain substances ("proselytizing")

In general, most SEs carried out in the fields of medicine seemed relatively simple and harmless (e.g., drawing blood, inserting a tube into the gastrointestinal tract, ventilation tests, etc.), and it appeared that these experiments have rarely resulted in significant damage to the experimenter (Altman 1986). The literature reviewed in this chapter suggests that virtually no serious physical complications have been reported, especially when the drugs in question were not taken on a regular basis. But there were exceptions from this rule, and a particular tragic and dramatic example could be seen in the neurotoxic effects induced by MPTP, a synthesis by-product found in the synthetic opioid MPPP, which led to irreversible precipitation of Parkinsonism in users exposed to this by-product (Langston 2017).

It is hard to estimate the right dose when newly synthesized substances are explored (Shulgin et al. 1986). On an individual level, risks of adverse effects are typically dose-dependent, but both "set" and "setting" are particularly important when working with substances such as LSD, psilocybin, or DMT that, under unsupportive conditions, carry the risk of eliciting traumatic experiences in the individual, thus presenting potential dangers during the acute phase of the inebriation.

Other complications such as unrealistic behavior can usually be limited within controlled and medically equipped environments. As illustrated by the cases of Crowley, Lilly, and Moore, the dynamics of self-experimentation might go beyond originally set limits that might even endanger the experimenter. Another difficulty can manifest in the development of a hypocritical attitude that can also take the form

of "proselytizing," thereby posing risks to others. A more serious form is the fixation on drug effects that lead to feelings of megalomania, sometimes triggered by certain specific drugs (e.g., cocaine or ketamine), and the dependence-producing substance cocaine led some investigators even to become "enslaved" by them (Crowley [1917] 1973; Ring 1887).

An example for some of these dangers can be found in the "Los Angeles group." These were highly qualified psychotherapists who began their therapeutic use of LSD and SEs in the late 1950s. In 1957, Sidney Cohen (of the Los Angeles Neuropsychiatric Institute) ordered LSD for the purpose of legitimate scientific experimentation. However, some of his associates became quite fascinated by the drug's effects and began to experiment on themselves on weekends. By this time, a chain of enthusiastic discovery extended from one researcher to another, which changed the group dynamics to a stage where drug taking itself became the center of attention. As a safety measure, these researchers developed a "buddy system" by which one partner took LSD while the other, abstaining, watched his performance and somewhat guided the experience. The increasing enthusiasm soon extended to include other substances and the establishment of "LSD social parties." Ultimately, the therapists' LSD supplies were confiscated in 1962 (Novak 1997; Caldwell 1968, pp. 47–49).

8 Conclusions

8.1 What Can Be Learned from the History of Self-Experimentation?

A synoptic view on the history of SEs with psychoactive substances leads to the recognition that the pleasures and risks associated with experiencing adverse effects differ regarding context and substances used. For example, it seems that the hallucinogens did not lead to immediate adverse effects when taken under controlled conditions, and they also did not induce behavior associated with dependence. The experiences induced by them have reportedly led to a deeper understanding of patients with psychotic illnesses and neurotic patients within the confines of LSD-assisted psychotherapy. With the recently upcoming new therapeutic methods for the effective treatment of post-traumatic stress disorder (PTSD) by MDMA-assisted psychotherapy (Mithoefer et al. 2011, 2013, 2018) and the use LSD in anxiety disorders (Gasser et al. 2014) and of psilocybin for depression (Carhart-Harris et al. 2016), one might even see a revival of SEs as an important requirement for training therapists who employ these methods.

Under certain circumstances, as seen with some studies and SEs in the past, it may appear that there was no alternative available when exploring new terrain in order to avoid posing risk to others. A notable example is Alexander Shulgin who was possibly one of the greatest self-experimenters and who remained lucid and healthy after nearly 50 years of such research. Even with this case in mind, the risks should not be underestimated and have to be evaluated for every substance in its own

right. This might be particularly relevant today in the world of NPS that might pose high risks to people who use these substances given that toxicological data are commonly unavailable.

Psychologists like William James and Sigmund Freud or philosophers like Benjamin P. Blood have been inspired significantly by their SEs. However, others have been confronted with serious dangers when their self-experimentation got out of control, especially so with substances with more simple and reliable euphoric effects that also carry a higher dependence liability. With such substances, the specific properties of the substances have to be considered in advance. For example, is it more a reliable euphoria-inducing stimulant or is it having unpleasant side effects? What do the animal experiments show in this respect? Does the substance induce "consciousness-expanding" qualities that elicit more intense and complex feelings and thoughts than usual that go beyond the users' usual frame of reference? If this is the case, then documented self-experimenters have tended to shy away from experiencing drug effects under crude and less favorable circumstances. This differs from other drugs such as cocaine, which tend to induce a "simple but reliable state of euphoria," ego-strengthening, and anxiolysis (cf. Table 1). Substances such as cocaine or certain amphetamine-like stimulants, which primarily engage the "reward systems" of the brain, might carry particular health risks through repeated use and/or dependence liability. It can be assumed that goals of self-experimentation serving other functions, such as escapism, manipulation, or psychological coping, are rarely communicated. One exception is the retrospective account of John C. Lilly's ketamine dependence, which began as a SE (Lilly 1978).

Another important aspect is the psychological state of the experimenter. Not all motives are known consciously or in advance. For example, a need for compensating for psychological deficits will predict a preference for substances with properties that allow for such compensation to take place, e.g., euphoric stimulants to cope with depressive feelings; opiates to cope with hyperarousal, depression, and nightmares; and benzodiazepines to cope with anxiety. In contrast, substances with more "complex" or even "consciousness-expanding" effects are not particularly usable for the purpose of coping with psychiatric symptoms. Instead of suppressing psychiatric symptoms or compensating psychological deficits, these substances tend to confront the drug takers with their deficits instead of aiding suppression or compensation.

If SEs appear unavoidable or necessary, it is advantageous for a researcher (or therapist) to work in the framework of controlled SEs where environmental circumstances are carefully controlled and characteristics of the substance used (as well as sufficient toxicological information) are known. These SEs can provide sufficient safety and a more reliable outcome, documentation, and instruction (if used by future researchers or therapists). In contrast, uncontrolled SEs might provide less scientific value and have repeatedly led to "unconventional behavior," social withdrawal, and autistic individual or group behavior. A positive example of serious and safe self-experimentation could be seen in the Swiss Physicians Society for Psycholytic Therapy (SÄPT). In its professional framework, more than 50 physicians have self-experimented under orderly and safe conditions for more than 30 years and did not produce any adverse effects (Gasser 2017, Personal

communication, Styk 1994). Other examples of safely controlled SEs were those conducted in clinical treatment centers where LSD therapy was practiced, which never resulted in grave complications (e.g., Winkler and Csémy 2014).

In summary, it appears that self-experimentation with psychoactive substances has, besides a continuous history for over 125 years, stimulated scientific (and therapeutic) advances. However, examples also exist that might serve as cautionary tales involving a variety of potentially dangerous dynamics, be it on an individual or group level.

As recent scientific and ethical restrictions do not allow for much scientifically driven SEs anymore, one can assume that the great times of undertaking controlled SEs appear to be over. Safely controlled SEs might find their legitimate place in the future in the training of therapists and the education of experimental researchers. As the last 15 years have shown, the future might see a further expansion of the spectrum and range of NPS and "self-experimentation" with them by curious laypersons, "para-professional" experimenters, or users with drug dependence. This type of drug taking might not be influenced by existing legislative control. During the last 10 years, it has consistently been argued that attempts to prohibit most psychoactive substances have led to the emergence of "new," and sometimes more harmful, successors. The easily foreseeable (and probably chaotic and dangerous) experimentation with NPS of the future might be restricted to the "wild" category performed by nonscientists, thus limiting safety and gains in scientific knowledge. With this in mind, it appears even more important what Altman (1972, p. 351) has concluded in his study on medical self-experimentation: ". . . The mere act of doing the experiment on oneself justifies neither a poorly designed experiment nor the same well designed experiment on someone else" (Altman 1972, p. 351).

References

Abouchedid R, Gilks T, Dargan PI, Archer JRH, Wood DM (2018) Assessment of the availability, cost, and motivations for use over time of the new psychoactive substances-benzodiazepines diclazepam, flubromazepam, and pyrazolam-in the UK. J Med Toxicol 14:134–143

Abramson HA (ed) (1967) The use of LSD in psychotherapy and alcoholism. Bobbs-Merrill, Indianapolis

Alberts L (1921) Einwirkungen des Meskalins auf komplizierte psychische Vorgänge. Dissertation, Heidelberg University, Heidelberg

Alles G (1959) Subjective reactions to phenethylamine hallucinogens. In: Featherstone RM, Simon A (eds) A pharmacologic approach to the study of the mind. C.C. Thomas, Springfield, pp 238–250

Altman LK (1972) Auto-experimentation – an unappreciated tradition in medical science. N Eng J Med 286:346–352

Altman LK (1986) Who goes first? The story of self-experimentation in medicine. Random House, New York

Ameline A, Richeval C, Gaulier JM, Raul JS, Kintz P (2018) Characterization of flunitrazolam, a new designer benzodiazepine, in oral fluid after a controlled single administration. J Anal Toxicol 42:e58–e60

Ammon G, Patterson PGR (1971) Peyote: Zwei verschiedene Ich-Erfahrungen. Dyn Psychiatr 4(Supplement 1):47–71

Anderson GM, Braun G, Braun U, Nichols DE, Shulgin AT (1978) Absolute configuration and psychotomimetic activity. In: Barnett G, Trsic M, Willette R (eds) QuaSAR – quantitative structure–activity relationships. NIDA research monograph, vol 22. United States Department of Health, Education, and Welfare, Washington, pp 8–15

Arnold OH, Hoff H (1953) Untersuchungen über die Wirkungsweise von Lysergsäurediäthylamid. Wien Z Nervenheilkd Grenzgeb 6:129–150

Arthur JD (2008) Peopled darkness: perceptual transformation through Salvia divinorum. iUniverse, New York

Assi S, Gulyamova N, Kneller P, Osselton D (2017) The effects and toxicity of cathinones from the users' perspectives: a qualitative study. Hum Psychopharmacol 32:e2610

Auwärter V, Dresen S, Weinmann W, Muller M, Putz M, Ferreiros N (2009) 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? J Mass Spectrom 44:832–837

Baumann MH, Glennon RA, Wiley JL (2017) Neuropharmacology of new psychoactive substances (NPS). In: The science behind the headlines. Springer, Cham

Becker AM (1949) Zur Psychopathologie der Lysergsäurediäthylamidwirkung. Wiener Z Nervenhk 2:402–407

Beecher HK (1959) Experimentation in man. J Am Med Assoc 169:461–478

Benz E (1989) Halluzinogen-unterstützte Psychotherapie. Medical dissertation, University of Zürich, Zürich

Benzenhöfer U, Passie T (2010) Rediscovering MDMA (ecstasy): the role of the American chemist Alexander T. Shulgin. Addiction 105:1355–1361

Beringer K (1923) Experimentelle Psychosen durch Mescalin. Z ges Neurol Psychiatr 84: 426–433

Beringer K (1927) Der Meskalinrausch. Springer, Berlin

Beringer K (1932) Zur Klinik des Haschischrausches. Nervenarzt 5:337–342

Bisbort A (2009) Beatniks: a guide to an American subculture. Greenwood, Westport

Brandt SD, King LA, Evans-Brown M (2014) The new drug phenomenon. Drug Test Anal 6: 587–597

Bumke O (1903) Scopolaminum hydrobromicum. Monatsschr Psychiatr Neurol 13:62–69

Caldwell WV (1968) LSD psychotherapy: an exploration of psychedelic and psycholytic therapy. Grove Press, New York

Calton T, Ferriter M, Huband N, Spandler H (2008) A systematic review of the Soteria paradigm for the treatment of people diagnosed with schizophrenia. Schizophr Bull 34:181–192

Carhart-Harris RL, Bolstridge M, Rucker J, Day CM, Erritzoe D, Kaelen M, Bloomfield M, Rickard JA, Forbes B, Feilding A, Taylor D, Pilling S, Curran VH, Nutt DJ (2016) Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. Lancet Psychiatry 3:619–627

Castiglioni A (1947) A history of medicine. Alfred A. Knopf, New York

Cohen S (1964) The beyond within the L.S.D. story. Atheneum, New York, pp 106–111

Cohen S (1967) Discussion. In: Abramson HA (ed) The use of LSD in psychotherapy. Josiah Macy, New York, p 11

Condrau G (1949) Klinische Erfahrungen an Geisteskranken mit Lysergsäure-Diäthylamid. Acta Psychiatr Scand 24:9–32

Crowley A ([1917] 1973) Cocaine. Level Press, San Francisco

Dargan PI, Wood DM (eds) (2013) Novel psychoactive substances. Academic Press, Amsterdam

Davy H (1800) Researches, chemical and philosophical: chiefly concerning nitrous oxide, or diphlogisticated nitrous air, and its respiration. J. Johnson, London

Downing JJ (1965) Zihuatanejo: an experience of transpersonative living. In: Blum R (ed) Utopiates – the use and users of LSD. Tavistock Publications, London, pp 142–178

Dulchinos DP (1999) Fitz Hugh Ludlow: pioneer of inner space. In: Lyttle T (ed) Psychedelics reimagined. Autonomedia, New York, pp 29–40

Ellis H (1898) Mescal, a new artificial paradise. Contemp Theatr Rev 73:131–141

Erowid E, Erowid F (2015) The kratom experience from firsthand reports. In: Raffa RB (ed) The chemistry and pharmacology of opioids from a non-opium source. CRC Press, Boca Raton, pp 213–244
European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2015) New psychoactive substances in Europe. An update from the EU early warning system. http://www.emcdda.europa.eu/system/files/publications/65/TD0415135ENN.pdf. Accessed 1 May 2018
Evans-Brown M, Sedefov R (2018) Responding to new psychoactive substances in the European Union: early warning, risk assessment, and control measures. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_160
Feldman HW, Agar MH, Beschner GM (eds) (1979) Angel dust. An ethnographic study of phencyclidine users. Lexington Books, Lexington
Ferkel S (1954) "Hexensalben" und ihre Wirkung. Kosmos 50:414–415
Fischer R (1946) Selbstbeobachtungen im Meskalinrausch. Schweiz Z Psychol 5:308–313
Fränkel F, Joël E (1927) Beiträge zu einer experimentellen Psychopathologie: der Haschischrausch. Z ges Neurol Psychiatr 111:84–106
Frederking W (1955) Intoxicant drugs (mescaline and lysergic acid diethylamide) in psychotherapy. J Nerv Ment Dis 121:262–266
Freud S (1884) Über Coca. Centralbl ges Ther 2:289–314
Freud S (1885a) Beitrag zur Kenntnis der Coca-Wirkung. Wien Med Wochenschr 5:129–133
Freud S (1885b) Zur Coca-Wirkung beim gesunden Menschen. Centralbl ges Ther 3:253–254
Friedrichs H ([1940] 2009) Die Psychologie des Meskalinrausches. VWB, Berlin
Gasser P, Holstein D, Michel Y, Doblin R, Yazar-Klosinski B, Passie T, Brenneisen R (2014) Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. J Nerv Ment Dis 202:513–520
Gonzalez D, Riba J, Bouso JC, Gomez-Jarabo G, Barbanoj MJ (2006) Pattern of use and subjective effects of Salvia divinorum among recreational users. Drug Alcohol Depend 85:157–162
Grof S (1964) K problematice optických poruch v autoexperimentech s halucinogeny. Česk Psychiatr 3:173–181
Grof S (1980) LSD psychotherapy. Hunter House, Pomona
Guttmann (1921) Hallluzinationen und andere Folgeerscheinungen nach experimenteller Vergiftung mit Anhalonium (Mescal). Berl Klin Wochenschr 58:816–817
Haldane JS (1922) Respiration. Yale University Press, New Haven
Haldane JS, Smith JL (1893) The physiological effects of air ventilated by respiration. J Pathol Bacteriol 1:168–186
Hearne E, Van Hout MC (2016) "Trip-sitting" in the black hole: a netnographic study of dissociation and indigenous harm reduction. J Psychoactive Drugs 48:233–242
Heffter A (1894) Über Pellote. Arch Exp Pathol Pharmakol 34:65–86
Heffter A (1898) Über Pellote. Zweite Mitteilung. Arch Exp Pathol Pharmakol 40:385–429
Heimann H (1952) Die Scopolaminwirkung. Karger, Basel
Henderson GL (1988) Designer drugs: past history and future prospects. J Forensic Sci 33:569–575
Henderson L, Glass W (eds) (1994) LSD: still with us after all these years. Lexington Books, New York
Hoffer A, Osmond H (1967) The hallucinogens. Academic Press, New York
Hofmann A (1955) The history of LSD-25. Triangle 2:117–124
Hofmann (1979) LSD-mein Sorgenkind. Klett-Cotta, Stuttgart
Hollingshead M (1973) The man who turned on the world. Blond & Briggs, London
Huppertz LM, Moosmann B, Auwärter V (2018) Flubromazolam – basic pharmacokinetic evaluation of a highly potent designer benzodiazepine. Drug Test Anal 10:206–211
Hutter M, Moosmann B, Kneisel S, Auwärter V (2013) Characteristics of the designer drug and synthetic cannabinoid receptor agonist AM-2201 regarding its chemistry and metabolism. J Mass Spectrom 48:885–894
Huxley A (1954) The doors of perception. Chatto & Windus, London
Huxley A (1980) Moksha. Chatto & Windus, London

James W (1882) Subjective effects of nitrous oxide. Mind 7:186–208
James W (1902) The varieties of religious experience. Longmans, New York
Jansen KL (2000) A review of the nonmedical use of ketamine: use, users and consequences. J Psychoactive Drugs 32:419–433
Jansen K ([2001] 2004) Ketamine: dreams and realities. MAPS, Sarasota
Jansen KL, Darracot-Cankovic R (2001) The nonmedical use of ketamine, part two: a review of problem use and dependence. J Psychoactive Drugs 33:151–158
Jay M (2009) The atmosphere of heaven. Yale University Press, London
Jöel E, Fränkel F (1924) Der Cocainismus. Ein Beitrag zur Geschichte und Psychopathologie der Rauschgifte. Springer, Berlin
Jöel E, Fränkel F (1926) Der Haschisch-Rausch. Beiträge zu einer experimentellen Psychopathologie. Klin Wochenschr 5:1707–1709
Joël E, Fränkel F (1929) Beiträge zur experimentellen Psychopathologie. Klin Wochenschr 8:549–550
Kant F, Kropf E (1928) Über Selbstversuche mit Haschisch. Naunyn Schmiedebergs Arch Exp Pathol Pharmakol 129:319–338
Kelly K (1999) The little book of ketamine. Ronin, Berkeley
Ketchum J (2006) Chemical warfare secrets almost forgotten. Chembooks, Santa Rosa
King LA (2014) New phenethylamines in Europe. Drug Test Anal 6:808–818
King LA, Kicman AT (2011) A brief history of 'new psychoactive substances'. Drug Test Anal 3: 401–403
Kintz P, Richeval C, Jamey C, Ameline A, Allorge D, Gaulier JM, Raul JS (2017) Detection of the designer benzodiazepine metizolam in urine and preliminary data on its metabolism. Drug Test Anal 9:1026–1033
Kirsch MM (1986) Designer drugs. CompCare Publications, Minneapolis
Kjellgren A, Jonsson K (2013) Methoxetamine (MXE) – a phenomenological study of experiences induced by a "legal high" from the internet. J Psychoactive Drugs 45:276–286
Kjellgren A, Soussan C (2011) Heaven and hell – a phenomenological study of recreational use of 4-HO-MET in Sweden. J Psychoactive Drugs 43:211–219
Kjellgren A, Henningsson H, Soussan C (2013) Fascination and social togetherness-discussions about spice smoking on a Swedish internet forum. Subst Abuse 7:191–198
Klinke O (1889) Hyoscin bei Geisteskranken. Z Nervenheilkd Psychopathol 12:189–196
Klüver H (1966) Mescal and mechanisms of hallucinations. University of Chicago Press, Chicago
Klüver H (1980) Foreword. In: Schultes RE, Hofmann A (eds) The botany and chemistry of hallucinogens, 2nd edn. C.C. Thomas, Springflied, pp VII–VVX
Knauer A, Maloney WJ (1913) A preliminary note on the psychic action of mescaline, with special reference to the mechanism of visual hallucinations. J Nerv Ment Dis 40:425–436
Konrad E (1888) Zur physiologischen und therapeutischen Wirkung des Hyoscinum hydrochloricum. Centralbl Nervenheilk 11:529
Langston JW (2017) The MPTP story. J Parkinsons Dis 7:S11–S22
Leary T (1966) Programmed communication during experiences with DMT (dimethyltryptamine). Psychedelic Rev 8:83–95
Leary T, Litwin GH, Metzner R (1963) Reactions to psilocybin administered in a supportive environment. J Nerv Ment Dis 137:561–573
Lewin L (1924) Phantastica. Die betäubenden und erregenden Genussmittel. Für Ärzte und Nichtärzte. Verlag von Georg Stilke, Berlin
Lewin L (1888) Über Anhalonium lewinii. Arch Exp Pathol Pharmakol 24:401–411
Lewin L (1998) Phantastica: a classic survey on the use and abuse of mind-altering plants. Park Street Press, Rochester
Lilly JC (1972a) Programming and metaprogramming in the human biocomputer. Julian Press, New York
Lilly JC (1972b) The center of the cyclone. Julian Press, New York
Lilly JC (1978) The scientist. Crown Publishers, New York

Ludlow F (1857) The Hasheesh eater. Harper & Bros, New York
Mantegazza P (1859 [1973]) Sulle virtu igienische e medicale de la coca. Ann univers di medic Bd. 167:449ff. Partially reprinted. In: Andrews G, Solomon D (eds) The coca leaf and cocaine papers. Harcourt Brace Jovanovich, New York, pp 38–42
Maqueda AE, Valle M, Addy PH, Antonijoan RM, Puntes M, Coimbra J, Ballester MR, Garrido M, Gonzalez M, Claramunt J, Barker S, Johnson MW, Griffiths RR, Riba J (2015) Salvinorin-A induces intense dissociative effects, blocking external sensory perception and modulating interoception and sense of body ownership in humans. Int J Neuropsychopharmacol 18:pyv065
Matussek N (1952) Wahnsymptome im Meskalinrausch. Medical dissertation, Max-Planck-Institut für Psychiatrie, München
Mayer-Gross W, McAdam W, Walker JW (1951) Psychological and biochemical effects of lysergic acid diethylamide. Nature 168:827–828
McGlothlin W, Cohen S, McGlothlin MS (1967) Long lasting effects of LSD on normals. Arch Gen Psychiatry 17:521–532
McKenna T (1991) The archaic revival. Speculations on psychedelic mushrooms, the amazon, virtual reality, UFOs, evolution, shamanism, the rebirth of the goddess, and the end of history. Harper Collins, San Francisco
Meyer P (1993) Apparent communication with discarnate entities related to DMT. Psychedelic Monogr Essays 6:29–67
Meyer MR (2018) Toxicokinetics of NPS: update 2017. Handb Exp Pharmacol. https://doi.org/10.1007/1164_2018_1102
Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, Doblin R (2011) The safety and efficacy of ±3,4-methylenedioxymethamphetamine-assisted psychotherapy in subjects with chronic, treatment-resistant posttraumatic stress disorder: the first randomized controlled pilot study. J Psychopharmacol 25:439–452
Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, Martin SF, Yazar-Klosinski B, Michel Y, Brewerton TD, Doblin R (2013) Durability of improvement in post-traumatic stress disorder symptoms and absence of harmful effects or drug dependency after 3,4-methylenedioxymethamphetamine-assisted psychotherapy: a prospective long-term follow-up study. J Psychopharmacol 27:28–39
Mithoefer MC, Mithoefer AT, Feduccia AA, Jerome L, Wagner M, Wymer J, Holland J, Hamilton S, Yazar-Klosinski B, Emerson A, Doblin R (2018) 3,4-Methylenedioxymethamphetamine (MDMA)-assisted psychotherapy for post-traumatic stress disorder in military veterans, firefighters, and police officers: a randomised, double-blind, dose-response, phase 2 clinical trial. Lancet Psychiatry 5:486–497
Moore M, Alltounian HS (1978) Journeys into the bright world. Para Research, Rockport
Moosmann B, Huppertz Laura M, Hutter M, Buchwald A, Ferlaino S, Auwärter V (2013a) Detection and identification of the designer benzodiazepine flubromazepam and preliminary data on its metabolism and pharmacokinetics. J Mass Spectrom 48:1150–1159
Moosmann B, Hutter M, Huppertz LM, Ferlaino S, Redlingshöfer L, Auwärter V (2013b) Characterization of the designer benzodiazepine pyrazolam and its detectability in human serum and urine. Forensic Toxicol 31:263–271
Moosmann B, Bisel P, Auwärter V (2014) Characterization of the designer benzodiazepine diclazepam and preliminary data on its metabolism and pharmacokinetics. Drug Test Anal 6:757–763
Morris H, Wallach J (2014) From PCP to MXE: a comprehensive review of the non-medical use of dissociative drugs. Drug Test Anal 6:614–632
Mosher L (1999) Lecture on Soteria treatment for psychotic patients. Hannover Medical School, Hannover
Nichols DE (2016) Psychedelics. Pharmacol Rev 68:264–355
Novak SJ (1997) LSD before Leary. Sidney Cohen's critique of 1950s psychedelic drug research. Isis 88:87–110

Osmond H (1970) Peyote night. In: Aaronson BS, Osmond H (eds) Psychedelics. The Hogarth Press, London, pp 67–86
Oss OT, Oeric ON (1976) Psilocybin – magic mushroom grower's guide. And/Or Press, Berkeley
Ott J (1994) Ayahuasca analogues–Pangean entheogens. Natural Products, Washington
Ott J (1999) Pharmahuasca: human pharmacology of oral DMT plus harmine. J Psychoactive Drugs 31:171–177
Ott J (2001a) Shamanic snuffs or entheogenic errhines. Entheobotanica, Solothurn
Ott J (2001b) Pharmañopo-psychonautics: human intranasal, sublingual, intrarectal, pulmonary and oral pharmacology of bufotenine. J Psychoactive Drugs 33:273–281
Ott J (2001c) Pharmepéna-psychonautics: human intranasal, sublingual and oral pharmacology of 5-methoxy-N,N-dimethyl-tryptamine. J Psychoactive Drugs 33:403–407
Passie T (1997) Hanscarl Leuner: pioneer of hallucinogen research and psycholytic therapy. Multidiscipl Assoc Psychedelic Stud Bull 7:46–49
Passie T (2002) Protocol for a research study of psilocybin in a clinical setting. Hannover Medical School, Hannover
Passie T (2005) Meskalin in Deutschland 1912–1945: Grundlagenforschung, Selbstversuche und Missbrauch. In: Pieper W (ed) Nazis on speed, vol 2, pp 234–244
Passie T (2018) The early use of MDMA ('Ecstasy') in psychotherapy (1977–1985). Drug Sci Policy Law 4:1–19
Passie T, Benzenhöfer U (2016) The history of MDMA as an underground drug in the United States, 1960–1979. J Psychoactive Drugs 48:67–75
Passie T, Benzenhöfer U (2018) MDA, MDMA and other "mescaline-like" substances in the US military's search for a truth drug (1940s to 1960s). Drug Test Anal 10:72–80
Pickering GW (1949) The place of experimental method in medicine. Proc R Soc Med 42:229–234
Prentiss DW, Morgan FP (1895) Anhalonium lewinii (Mescal buttons): a study of the drug with special reference to its physiological action upon man with report of experiments. Therapeut Gaz 19:577–585
Prinzhorn H (1927) Erdentrückte Seele. Leuchter 8:277–296
Prinzhorn H (1928) Entrückung durch Rauschgift. Zeitsch Parapsychol 55:24–34
Resnikoff C (2018) Mail to Torsten Passie. 6 Apr 2018
Ring FW (1887) Cocaine and its fascinations, from a personal experience. Medical Record, September 3, 1887. In: Andrews G, Solomon D (eds) The coca leaf and cocaine papers, vol 1973. Harcourt Brace Jovanovich, New York, pp 253–258
Roubíček J (1961) Experimentální psychosy. SZdN, Prague
Shedlin M, Wallechinski D, Sayler S (eds) ([1973] 1992) Laughing gas. Ronin, Berkeley
Shulgin AT (1975) Centrally active phenethylamines. Psychopharmacol Commun 1:93–98
Shulgin A, Shulgin A (1991) PIHKAL. Transform Press, Berkeley
Shulgin A, Shulgin A (1997) TIHKAL. Transform Press, Berkeley
Shulgin AT, Shulgin LA, Jacob PA (1986) A protocol for the evaluation of new psychoactive drugs. Methods Find Exp Clin Pharmacol 8:313–320
Siebert DJ (1994) Salvia divinorum and Salvinorin A – new pharmacological findings. J Ethnopharmacol 43:53–56
Simpson ESC, Weiner JA (eds) (1989) The Oxford encyclopaedic English dictionary. Clarendon Press, Oxford
Solms H (1956) Relationships between chemical structure and psychoses with the use of psychotoxic substances. "Comparative pharmacopsychiatric analysis": a new research method. J Clin Exp Psychopathol 17:429–433
Solomon D (1964) Editor's preface. In: Solomon D (ed) LSD – the consciousness-expanding drug. Putnam's, New York, pp IX–IX
Soussan C, Kjellgren A (2014) Harm reduction and knowledge exchange – a qualitative analysis of drug-related internet discussion forums. Harm Red J 11:25–33
Soussan C, Kjellgren A (2015) "Chasing the high" – experiences of ethylphenidate as described on international internet forums. Subst Abuse 9:9–16

Soussan C, Andersson M, Kjellgren A (2018) The diverse reasons for using novel psychoactive substances – a qualitative study of the users' own perspectives. Int J Drug Policy 52:71–78
Stafford P (1990) Magic grams: investigations into psychedelic consciousness with Dr. Oscar Janiger. Psychedelic Monogr Essays 5
Stolaroff M (1994) Thanatos to Eros: thirty-five years of psychedelic exploration. VWB, Berlin
Stolaroff M (2004) The secret chief revealed. MAPS, Sarasota
Stoll WA (1947) Lysergsäure-diäthylamid, ein Phantastikum aus der Mutterkorngruppe. Schweiz Arch Neurol Psychiat 60:279–280
Styk J (1994) Rückblick auf die letzten sieben Jahre der Schweizerischen Ärztegesellschaft für Psycholytische Therapie (SÄPT). In: Dittrich A, Hofmann A, Leuner H (eds) Welten des Bewusstseins, vol 4. VWB, Berlin, pp 149–154
Swogger MT, Hart E, Erowid F, Erowid E, Trabold N, Yee K, Parkhurst KA, Priddy BM, Walsh Z (2015) Experiences of Kratom users: a qualitative analysis. J Psychoactive Drugs 47:360–367
Szára S (1957) The comparison of the psychotic effect of tryptamine derivatives with the effects of mescaline and LSD-25 in self-experiments. In: Garattini S, Ghetti V (eds) Psychotropic drugs. Elsevier, Amsterdam, pp 460–467
Szára S (1961) Hallucinogenic effects and metabolism of tryptamine derivatives in man. Fed Proc 20:885–888
Tettey JNA, Crean C, Ifeagwu SC, Raithelhuber M (2018) Emergence, diversity and control of new psychoactive substances – a global perspective. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_XXX
Trachsel D (2011) Psychedelische chemie. Nachtschatten Verlag, Solothurn
Trachsel D (2012) Fluorine in psychedelic phenethylamines. Drug Test Anal 4:577–590
Trachsel D, Lehmann D, Enzensperger C (2013) Phenethylamine: Von der Struktur zur Funktion. Nachtschatten Verlag, Solothurn
Turner DM (1996) Salvinorin – the psychedelic essence of Salvia divinorum. Panther Press, San Francisco
United Nations Office on Drugs and Crime (UNODC) (2018) Understanding the synthetic drug market: the NPS factor. https://www.unodc.org/documents/scientific/Global_Smart_Update_2018_Vol.19.pdf. Accessed 29 May 2018
Valdes LJ, Diaz JL, Paul AG (1982) Ethnopharmacology of Ska Maria Pastora (Salvia divinorum Epling and Jativa-M.). J Ethnopharmacol 7:287–312
Van Dusen W (1961) LSD and the enlightenment of Zen. Psychologia 4:11–16
Van Hout MC (2014) An internet study of user's experiences of the synthetic cathinone 4-methylethcathinone (4-MEC). J Psychoactive Drugs 46:273–286
Van Hout MC, Hearne E (2015a) "Plant or poison": a netnographic study of recreational use of 1,3-dimethylamylamine (DMAA). Int J Drug Policy 26:1279–1281
Van Hout MC, Hearne E (2015b) "Word of mouse": indigenous harm reduction and online consumerism of the synthetic compound methoxphenidine. J Psychoactive Drugs 47:30–41
Wagmann L, Maurer HH (2018) Bioanalytical methods for new psychoactive substances. Handb Exp Pharmacol. https://doi.org/10.1007/1164_2017_1083
Waser PG (1990) Psychiatrie, psychopharmaka und drogen in Zürich. Kommissionsverlag Beer AG, Zürich
Wasson RG (1962) Salvia divinorum – a new psychotropic drug from the mint family. Bot Mus Leafl Harvard Univ 20:77–84
Weil A (1976) MDA – the love drug. J Psychedelic Drugs 8:335–337
Weil A (1980) The marriage of the sun and the moon. Houghton Mifflin, Boston
Weir Mitchell S (1896) Remarks on the effects of Anhelonium lewinii (the mescal button). Br Med J 2:1625–1629
Wellen BJ (1986) Zur Geschichte des Bilsenkrautes. Dissertation, Fachbereich Pharmazie Philipps-Universität Marburg, Marburg
Weyl B (1951) Versuch einer psychopathologischen analyse der LSD-Wirkung. Medical dissertation, Albert-Ludwig-University Freiburg, Freiburg im Breisgau

Widmer S (1997) Ins Herz der Dinge Lauschen, 2nd edn. Nachtschatten, Solothurn
Winkler P, Csémy L (2014) Self-experimentations with psychedelics among mental health professionals: LSD in the former Czechoslovakia. J Psychoactive Drugs 46:11–19
Wood HC Jr (1869) On the medical activity of the hemp plant as grown in North America. Proc Am Philos Soc 11:226–232
Zucker K (1926) Die pharmakologische Betrachtungsweise als Forschungsmethode in der Neurologie und Psychiatrie. Klin Wochenschr 5:1977–1779

Neuropharmacology of Synthetic Cathinones

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Abstract

Synthetic cathinones are derivatives of the naturally occurring compound cathinone, the main psychoactive ingredient in the khat plant *Catha edulis*. Cathinone is the β-keto analog of amphetamine, and all synthetic cathinones display a β-keto moiety in their structure. Several synthetic cathinones are widely prescribed medications (e.g., bupropion, Wellbutrin®), while others are problematic drugs of abuse (e.g., 4-methylmethcathinone, mephedrone). Similar to amphetamines, synthetic cathinones are psychomotor stimulants that exert their effects by impairing the normal function of plasma membrane transporters for dopamine (DAT), norepinephrine (NET), and 5-HT (SERT). Ring-substituted cathinones like mephedrone are transporter substrates that evoke neurotransmitter release by reversing the normal direction of transporter flux (i.e., releasers), whereas pyrrolidine-containing cathinones like 3,4-methylenedioxypyrovalerone (MDPV) are potent transporter inhibitors that block neurotransmitter uptake (i.e., blockers). Regardless of molecular mechanism, all synthetic cathinones increase extracellular monoamine concentrations in the brain, thereby enhancing cell-to-cell monoamine signaling. Here, we briefly review the mechanisms of action, structure-activity relationships, and in vivo pharmacology of synthetic cathinones. Overall, the findings show that certain synthetic cathinones are powerful drugs of abuse that could pose significant risk to users.

Keywords

Cathinone · Dopamine · Monoamine · Serotonin · Stimulant · Transporter

Acronyms of the Discussed New Psychoactive Substances (NPS)

4-Bromo MCAT	1-(4-Bromophenyl)-2-(methylamino)propan-1-one (brephedrone)
4-Chloro MCAT	1-(4-Chlorophenyl)-2-(methylamino)propan-1-one (clephedrone)
4-Fluoro MCAT	1-(4-Fluorophenyl)-2-(methylamino)propan-1-one (flephedrone)
4-Methyl MCAT (4-MMC)	2-(Methylamino)-1-(4-methylphenyl)propan-1-one (mephedrone)
4-Methoxy MCAT	1-(4-Methoxyphenyl)-2-(methylamino)propan-1-one (methedrone)
4-TFM MCAT	2-(Methylamino)-1-[4-(trifluoromethyl)phenyl] propan-1-one
MCAT	2-(Methylamino)-1-phenylpropan-1-one (methcathinone)
MDMA	1-(2*H*-1,3-Benzodioxol-5-yl)-*N*-methylpropan-2-amine
MDMC	1-(2*H*-1,3-Benzodioxol-5-yl)-2-(methylamino) propan-1-one (methylone)

MDPV	1-(2*H*-1,3-Benzodioxol-5-yl)-2-(pyrrolidin-1-yl) pentan-1-one
α-PBP	1-Phenyl-2-(pyrrolidin-1-yl)butan-1-one
α-PHP	1-Phenyl-2-(pyrrolidin-1-yl)hexan-1-one
α-PPP	1-Phenyl-2-(pyrrolidin-1-yl)propan-1-one
α-PVP	1-Phenyl-2-(pyrrolidin-1-yl)pentan-1-one

1 Introduction

1.1 Synthetic Cathinones as Medications and Drugs of Abuse

Synthetic cathinones are chemical analogs of the naturally occurring compound cathinone, the main psychoactive ingredient in the khat plant *Catha edulis*. From a structural perspective, cathinone is the β-keto analog of amphetamine, and synthetic cathinones are often referred to as "bk-amphetamines" (see Fig. 1 for chemical structures). The stimulant effects of khat have been known for centuries, and the practice of chewing khat leaves is still popular today in many countries of East Africa and the Arabian Peninsula (Al-Hebshi and Skaugh 2005; Engidawork 2017). However, it was not until the 1970s that cathinone, specifically the (*S*)-(−) stereoisomer of cathinone, was isolated from khat leaves and identified as the primary psychoactive compound in the plant (Kalix 1990). Many years before the discovery of cathinone in khat, the compound was synthesized by medicinal chemists (e.g., Van der Schoot et al. 1962), and various derivatives have been investigated for therapeutic potential.

Figure 1 depicts the chemical structures of cathinone-related compounds that are prescribed medications approved by the Food and Drug Administration (FDA) in the USA. Diethylpropion, or amfepramone, is the *N,N*-diethyl analog of cathinone. Diethylpropion was developed as an anorectic agent in the early 1960s by the German pharmaceutical company Temmler-Werke (Schütte 1961) and is still prescribed today as Tenuate®, an efficacious adjunct for weight loss (Cercato et al. 2009; Suplicy et al. 2014). The pyrrolidine-containing cathinone analog, pyrovalerone, was investigated as an anti-fatigue agent in the 1960s (Wander 1963; Thomae 1963; Seeger 1967; Goldberg et al. 1973). Although pyrovalerone is an approved medication in the USA, it is rarely prescribed. Finally, bupropion is an *N-tert*-butyl analog of cathinone that was initially investigated as an antidepressant in the 1970s by Burroughs Wellcome (now GlaxoSmithKline) and subsequently approved for clinical use as Wellbutrin® (Mehta 1974; Dhillon et al. 2008). Bupropion was also approved as the smoking cessation aid Zyban® in 1997 (Dwoskin et al. 2006). In 2016, bupropion was the fifth most prescribed psychiatric medication in the USA (Grohol 2017).

While the cathinone-related compounds described above are used as efficacious medications, other synthetic analogs are misused as drugs of abuse. The *N*-methylated analog of cathinone, methcathinone, was a popular drug of abuse known as ephedrone

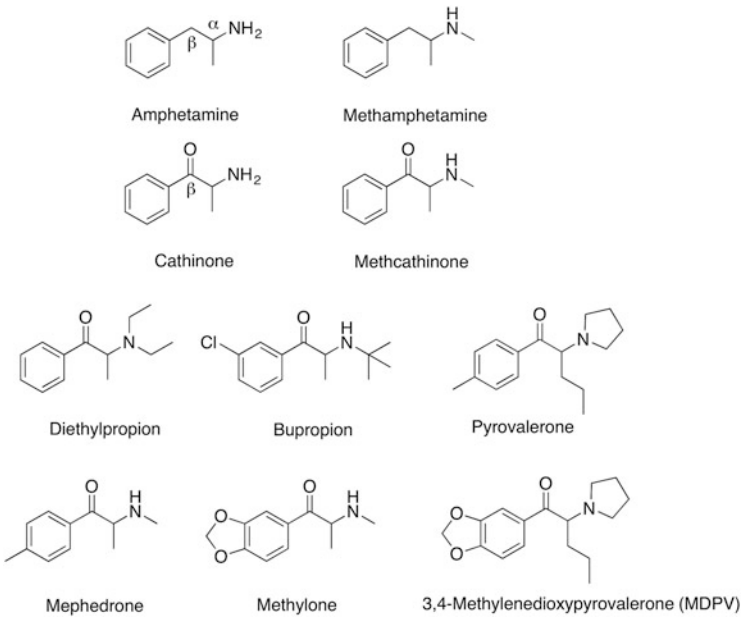

Fig. 1 Chemical structures of synthetic cathinones. Synthetic cathinones are β-keto amphetamines. Diethylpropion, bupropion, and pyrovalerone are FDA-approved medications in the USA, whereas mephedrone, methylone, and MDPV are abused drugs that were first encountered in so-called "bath salts" products

or "Jeff" in Russia during the 1980s (Emerson and Cisek 1993). Given that methcathinone is the β-keto analog of methamphetamine (see Fig. 1), it is not surprising that methcathinone induces powerful psychomotor stimulant effects and is known to show dependency-producing properties in animals and humans (Goldstone 1993; Kaminski and Griffiths 1994). An epidemic of methcathinone misuse occurred in the USA during the 1990s but quickly subsided. It is noteworthy that the chronic use of methcathinone was associated with the development of an irreversible Parkinsonian syndrome due to manganese toxicity (Stepens et al. 2008; Sikk and Taba 2015), secondary to the use of potassium permanganate as an oxidizing agent in the clandestine synthesis of the drug. In more recent times, a variety of "designer" synthetic cathinones have appeared in the nonmedical (i.e., recreational) drug market as substances of abuse (Baumann 2014; De Felice et al. 2014).

1.2 New Psychoactive Substances (NPS) and "Bath Salts" Cathinones

The abuse of psychomotor stimulants like methamphetamine and cocaine is a widespread public health problem that continues to plague modern society (Degenhardt et al. 2014). In this regard, a disturbing new trend is the increased recreational use of

so-called designer drugs, legal highs, or research chemicals (Baumann et al. 2014a; Madras 2017; Huestis et al. 2017). These drugs, collectively known as "new psychoactive substances" (NPS), are synthetic alternatives to more traditional drugs of abuse. NPS can be more formally defined as individual drugs in pure form or complex preparations that are not scheduled under the 1961 Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances but which might pose a public health threat (Madras 2017). Synthetic cathinones represent a predominant class of NPS (Baumann 2014). The first cathinone-related NPS in the USA were found in so-called "bath salts" products which were available in the recreational drug market during late 2010 (Prosser and Nelson 2012). By 2011, there were increasing reports of bath salts intoxications to poison control centers and emergency departments nationwide (Centers for Disease Control and Prevention 2011; Spiller et al. 2011; Warrick et al. 2013). Bath salts consist of powders or crystals that are administered intranasally, intravenously, or orally to produce their psychoactive effects. Low doses of bath salts produce typical stimulant effects such as increased energy, elevated mood and euphoria, but high doses or repeated use can induce serious symptoms including hallucinations, psychosis, tachycardia, hypertension, and hyperthermia, often accompanied by aggressive or violent behaviors (Banks et al. 2014; Karch 2015).

Forensic analysis of bath salts products in 2010 and 2011 identified three synthetic compounds: 4-methyl-*N*-methylcathinone (4-MMC, mephedrone), 3,4-methylenedioxy-*N*-methylcathinone (MDMC, methylone), and 3,4-methylene dioxypyrovalerone (MDPV) (Spiller et al. 2011; Shanks et al. 2012). Figure 1 depicts the chemical structures of the principal bath salts cathinones. Legislation passed in 2013 placed mephedrone, methylone, and MDPV into permanent Schedule I control, making the drugs illegal in the USA (DEA 2013). However, new cathinone derivatives are constantly being developed to circumvent legislative control, and by 2017 more than 100 cathinones had been identified worldwide (UNODC 2017). Most synthetic cathinones are manufactured by Asian chemical companies and sold over the Internet. Synthetic chemists consult the biomedical and patent literature for lead compounds to create novel analogs for recreational use. Given the increasing variety of synthetic cathinones in the recreational drug market, and the renewed interest in these compounds, the purpose of this chapter is to briefly review the molecular mechanisms of action, structure-activity relationships (SAR), and in vivo biological effects of synthetic cathinones.

2 Molecular Mechanisms of Action

2.1 Stimulant Drugs Target SLC6 Plasma Membrane Transporters

As noted above, synthetic cathinones are β-keto analogs of amphetamine (see Fig. 1). The bath salts drugs, mephedrone and methylone, have functional groups attached to their phenyl rings and are considered ring-substituted cathinones. MDPV has a more complex structure, with a bulky nitrogen-containing pyrrolidine ring and a flexible propyl chain extending from its α-carbon. Like other stimulant drugs, bath

salts cathinones exert their effects by interacting with plasma membrane transporter proteins expressed on nerve cells that synthesize the monoamine neurotransmitters dopamine, norepinephrine, and serotonin (5-HT) (Baumann et al. 2013; Eshleman et al. 2013; Simmler et al. 2013). In order to understand the molecular mechanism of action for cathinone analogs, it is essential to first consider the normal physiological role of monoamine transporters and the types of drugs targeting these proteins.

The solute carrier 6 (SLC6) transporters for dopamine (DAT), norepinephrine (NET), and serotonin (SERT) are responsible for translocating previously released neurotransmitter molecules from the extracellular space back into the neuronal cytoplasm, a process known as neurotransmitter "uptake" (Kristensen et al. 2011; Alexander et al. 2017). The uptake mechanism is a complex active-transport process dependent upon ionic gradients across neuronal membranes. Importantly, transporter-mediated neurotransmitter uptake is the principal mechanism for terminating the action of monoamine signaling, so drugs targeting these transporter proteins can have profound effects on cell-to-cell monoamine transmission. Monoamine transporters are the principal sites of action for medications used to treat a range of psychiatric diseases such as major depression, anxiety, and attention-deficit hyperactivity disorder (Iverson 2006; Sanchez et al. 2014; Faraone 2018). Drugs which preferentially interact at SERT, or 5-HT-selective reuptake inhibitors (SSRIs), are widely prescribed as treatments for major depression and anxiety disorders. By contrast, drugs which preferentially act at DAT and NET, such as amphetamine and methamphetamine, can have powerful psychomotor stimulant and dependence-producing properties (Rothman and Baumann 2003; Howell and Kimmel 2008; Espana and Jones 2013).

2.2 Transporter Blockers Versus Substrates

Drugs that bind to monoamine transporters can be divided into two types based on their precise mode of action: (1) cocaine-like "blockers" bind to the orthosteric site on the transporter and inhibit uptake of neurotransmitters from the extracellular space, whereas (2) amphetamine-like "substrates" also bind to the orthosteric site but are subsequently translocated through the transporter channel into the neuronal cytoplasm and trigger efflux of intracellular neurotransmitter molecules by reverse transport (i.e., transporter-mediated release) (Reith et al. 2015; Sitte and Freissmuth 2015). Drugs that act as transporter substrates are sometimes called transporter "releasers" because they induce non-exocytotic transporter-mediated release of neurotransmitters from neurons. Regardless of molecular mechanism, all drugs which interact with transporters can dramatically increase extracellular concentrations of monoamines in vivo, amplifying cell-to-cell chemical signaling throughout the central nervous system.

It is important to distinguish between transporter blockers and substrates because substrates display a number of unique properties: they are translocated into cells along with sodium ions, they induce inward depolarizing currents (Sonders et al. 1997; Sitte et al. 1998), and they reverse the normal direction of transporter flux to

trigger non-exocytotic release of neurotransmitters (i.e., reverse transport) (Hilber et al. 2005; Robertson et al. 2009). Finally, because substrate-type drugs are transported into cells, they can accumulate in the cytoplasm and interact with neuronal proteins to inhibit neurotransmitter synthesis and disrupt vesicular storage, leading to long-term neurotransmitter deficits (Fleckenstein et al. 2007; Baumann et al. 2014b). Table 1 summarizes some fundamental differences between transporter blockers versus substrates.

3 Methods to Study Transporter Function

The existence of monoamine transport mechanisms in cells was postulated long ago based on the ability of native tissues to take up and accumulate radiolabeled monoamines. For example, early studies characterized the accumulation of systemically administered [^{3}H]norepinephrine into mammalian tissues such as the heart, spleen, and liver (Axelrod et al. 1961). Other investigations showed that certain drugs, like cocaine and amphetamine, were able to inhibit the uptake of [^{3}H] neurotransmitters into nervous tissue, providing evidence for specific binding sites associated with transport mechanisms (Heikkila et al. 1975). The use of tissue homogenates, like synaptosomes, allowed for the possibility of studying transport mechanisms in situ in a high-throughput manner. Synaptosomes consist of sealed nerve endings with their plasma membrane leaflets oriented in a manner akin to neurons in vivo. Importantly, synaptosomes contain the full complement of protein machinery required for synthesis, metabolism, uptake, and exocytotic release of neurotransmitters.

Table 1 Comparison between the effects of monoamine transporter blockers versus substrates

Parameter	Monoamine transporter blockers	Monoamine transporter substrates (i.e., releasers)
Inhibit neurotransmitter uptake	Yes	Yes
Enter into neurons	No	Yes
Induce inward depolarizing Na^+ currents	No	Yes
Trigger reverse transport (transporter-mediated release)	No	Yes
Increase extracellular concentrations of transmitters	Yes	Yes
Neurochemical effects impulse- and TTX-sensitive	Yes	No
Neurochemical effects Ca^{++}- and reserpine-sensitive	Yes	No
Long-term neurotoxic deficits in monoamine neurons	No	Yes

3.1 Transporter Assays in Synaptosomes

Rothman and colleagues developed in vitro functional assays to assess the ability of test drugs to act as transporter blockers or substrates at DAT, NET, and SERT (Rothman et al. 2001, 2003a, b). We have adapted these methods in our laboratories and perform two types of assays: (1) uptake inhibition and (2) release stimulation. The assays are carried out in synaptosomes prepared from rat brain tissue and are designed to assess potency and efficacy of drugs at all three transporters under similar physiologically relevant conditions. For the uptake inhibition assays, radiolabeled substrate (i.e., [^{3}H]neurotransmitter) and test drug are co-incubated with synaptosomes for a brief period of time, and the reaction is stopped by vacuum filtration. If test drugs are transporter blockers, the accumulation of [^{3}H]neurotransmitter into synaptosomes (i.e., uptake) is reduced because the test drug and neurotransmitter compete for the same orthosteric site on the transporter protein. It is noteworthy that uptake inhibition assays cannot distinguish between blockers and substrates because both types of drugs will effectively inhibit the accumulation of [^{3}H]neurotransmitter into synaptosomes.

To identify substrate-type drugs, we use release stimulation assays. For the release assays, synaptosomes are first incubated with radiolabeled substrate molecules in order to fill or "preload" the interior of the synaptosomes. [^{3}H]1-Methyl-4-phenylpyridinium ([^{3}H]MPP$^+$) is used as the radiolabeled substrate for DAT and NET release assays, whereas [^{3}H]5-HT is used for SERT release assays. Once synaptosomes are preloaded, test drug is added for a brief incubation period, and the reaction is stopped by vacuum filtration. Drugs that act as transportable substrates will evoke efflux of [^{3}H]MPP$^+$ or [^{3}H]5-HT out of the synaptosomes (i.e., release) by reversal of the normal direction of transporter flux. Drugs that act as non-transportable blockers will not cause substantial release of [^{3}H]MPP$^+$ or [^{3}H]5-HT from preloaded synaptosomes. Thus, by testing drugs in the combined uptake inhibition and release assay procedures, the precise molecular mechanism of drug action can be ascertained. The data in Fig. 2 illustrate that amphetamine and cocaine both inhibit [^{3}H]dopamine uptake in synaptosomes, whereas only the substrate amphetamine is able to induce fully efficacious release of [^{3}H]MPP$^+$ via DAT.

3.2 Transporter Assays in Transfected Cells

The cloning of human isoforms of DAT, NET, and SERT in the 1990s (Pacholczyk et al. 1991; Giros et al. 1991; Shimada et al. 1991; Kilty et al. 1991) initiated a new era for evaluating the effects of psychostimulants and other drugs in heterologous expression systems (Eshleman et al. 1994; Pifl et al. 1995). The expression of cloned transporters in cells enabled the investigation of pure populations of a single transporter type in the absence of the synaptic protein machinery normally present in synaptosomes. Using cells transfected with DAT, NET, or SERT, it is possible to examine the effects of drugs on uptake and release of [^{3}H]neurotransmitters in a controlled and detailed manner. We have compared the pharmacological effects of

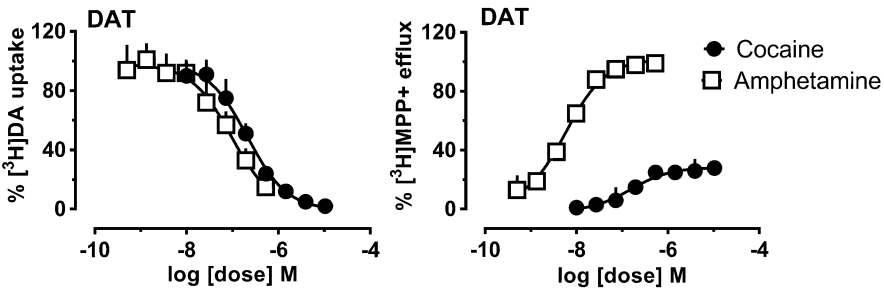

Fig. 2 Dose-response effects for cocaine and amphetamine in DAT uptake and release assays in rat brain synaptosomes. Data are depicted as mean ± SD for $N = 3$ experiments performed in triplicate. Note that cocaine and amphetamine both fully inhibit [^{3}H]dopamine uptake (left panel), whereas only the transporter substrate amphetamine evokes fully efficacious release of [^{3}H]MPP$^+$ (right panel)

many compounds in synaptosomes and human embryonic kidney 293 (HEK) cells stably expressing human monoamine transporters, to address possible differences in the results from these two approaches (Baumann et al. 2014b; Saha et al. 2015; Sandtner et al. 2016; Mayer et al. 2016). Overall, the findings demonstrate excellent agreement between synaptosomes and transporter-expressing cells in terms of identifying drugs as either transporter blockers or substrates. However, there are often discrepancies in absolute potency estimates for drugs (e.g., IC_{50} or EC_{50} values) in synaptosomes versus transfected cells. For example, the EC_{50} values for substrate-type drugs to evoke transporter-mediated release are often tenfold lower in synaptosomes when compared to cells expressing transporters. It also must be noted that the absolute amount of [^{3}H]neurotransmitter release can differ substantially across various assays, depending on the expression system used and specific transporter under examination.

3.3 Transporter-Associated Ionic Currents

From a mechanistic perspective, the transporter-mediated uptake of substrate molecules is best described by the "alternating access" model originally proposed by Jardetzky (1966) more than 50 years ago. The model posits that transporter proteins alternate between two distinct conformations: (1) an "outward-facing" conformation which has binding sites for substrate (e.g., dopamine) and co-substrate ions (e.g., Na^+, Cl^-) on the extracellular side of the protein and (2) an "inward-facing" conformation which has binding sites on the intracellular side and allows detachment of the substrate into the cytoplasm. The transition from outward-facing to inward-facing conformation is causally linked to movement of substrate molecules through the transporter. Additionally, the process of translocating substrates and their co-transported ions generates measurable ionic currents (Sonders and Amara 1996). Transporter-associated currents are a distinct property of the

proteins that resemble ion channel function, though the currents generated by transporters are much smaller than those generated by true ion channel proteins.

Transporter-mediated uptake of substrates is an active process that is fueled by the coupling of substrate flux to the movement of co-substrate ions down their electrochemical gradients. In particular, substrate translocation is dependent upon intact sodium gradients across cell membranes. The binding of substrate and co-substrate ions occurs in a fixed ratio, determined by the specific binding site topology of each transporter. Hence, the ion/substrate stoichiometry predicts the movement of a fixed number of electrical charges during every translocation cycle, whereby uptake of substrate will result in a net transmembrane current. Thus far, all transporters examined elicit inward positive current when translocating substrates, so they are considered electrogenic. It is noteworthy that SERT uses a counter-transported potassium ion to facilitate its return from the inward-facing to outward-facing conformation. The counter-transported potassium ion should render the transport cycle of SERT electroneutral, since 1 net-positive charge in (i.e., 1 Na^+, 1 5-HT^+ and 1 Cl^-) is canceled by the 1 positive K^+ charge out (Rudnick 1998). In contrast to this prediction, several studies show that SERT generates a positive inward current upon administration of 5-HT or other substrates (Mager et al. 1994; Adams and DeFelice 2003; Quick 2003; Hilber et al. 2005).

DAT and NET do not counter-transport potassium and, therefore, work in an electrogenic manner. Importantly, DAT, NET, and SERT display channel-like properties, since they allow the passage of ions "in excess" of the stoichiometric prediction, generally termed uncoupled conductance (Sonders et al. 1997; Sitte et al. 1998). Using voltage-clamp techniques in cells expressing transporter proteins, the ionic currents generated by cognate substrates (e.g., dopamine) and substrate-type drugs (e.g., amphetamine) can be accurately measured. It is now established that generation of transporter-associated currents is an inherent property of transporter substrates only, thus distinguishing transportable substrates from non-transportable blockers which do not induce currents (Schicker et al. 2012).

3.4 Effects of the Ionophore Monensin

As noted above, the transporter-mediated movement of substrate molecules is an energy-requiring process dependent upon intact ionic gradients across cell membranes. We have conducted experiments to examine transporter function under conditions where intracellular sodium concentrations are elevated by the addition of ouabain or monensin (Scholze et al. 2000; Sitte et al. 2000). Ouabain inhibits the activity of Na^+/K^+-ATPase to disrupt sodium gradients across cells, whereas monensin is an ionophore which facilitates transmembrane exchange of sodium ions for protons (Mollenhauer et al. 1990). It is noteworthy that a rise in intracellular sodium concentration greatly increases the propensity for outward transport and [^{3}H]neurotransmitter efflux via the transporter (Raiteri et al. 1978; Liang and Rutledge 1982; Bönisch 1986). We have used monensin as a tool to discriminate transporter substrates from blockers in release assays carried out in cells

expressing transporter proteins. Cells preloaded with [^{3}H]MPP$^+$ are incubated with transporter ligands in the presence or absence of monensin. Under these conditions, the efflux of [^{3}H]MPP$^+$ induced by transporter substrates is greatly enhanced in the presence of monensin, whereas the effects of transporter blockers are unaltered (Baumann et al. 2013; Mayer et al. 2016).

4 Structure-Activity Relationships

Structure-activity relationship (SAR) investigations examine the effects of altering the chemical structure of a given drug molecule on biological responses. In the simplest approach to SAR, one specific substituent on a drug molecule is altered, while the remainder of the molecule is "locked-in" and stays constant (Glennon and Dukat 2017). Employing SAR studies, it is possible to determine the role of a given chemical group in modulating the functional activity of candidate medications or drugs of abuse. Prior to the appearance of bath salts cathinones in 2010–2011, few scientific studies had examined the SAR for cathinone-related compounds. Evidence from the 1980s showed that cathinone and methcathinone release dopamine from rat brain tissue by an amphetamine-like mechanism (Kalix and Glennon 1986; Glennon et al. 1987), and subsequent reports demonstrated that methcathinone acted as a potent substrate at DAT and NET but not SERT (Cozzi et al. 1999; Rothman et al. 2003b). Cozzi et al. first reported that methylone acts as an uptake blocker at monoamine transporters (Cozzi et al. 1999), while other investigations showed the drug is a transporter substrate capable of releasing dopamine, norepinephrine, and 5-HT from rat brain tissue (Nagai et al. 2007). A number of more recent studies have characterized the SAR for ring-substituted and pyrrolidine-containing cathinones.

4.1 Ring-Substituted Cathinones Are Transporter Substrates

Hadlock et al. (2011) carried out the first comprehensive investigation of the pharmacology of the bath salts cathinone, mephedrone. These investigators found that mephedrone inhibits dopamine uptake and stimulates dopamine release from rat brain synaptosomes. López-Arnau et al. (2012) reported that mephedrone and methylone both inhibit uptake at DAT and SERT in synaptosomes, but no transporter release data were reported in their study. Our laboratory extended the findings of López-Arnau and coworkers by showing that mephedrone and methylone act as transporter substrates in rat brain synaptosomes, thereby evoking the release of [^{3}H]MPP$^+$ from DAT and NET and release of [^{3}H]5-HT from SERT (Baumann et al. 2012). The nonselective substrate activity of mephedrone and methylone at monoamine transporters is similar to the molecular mechanism of action for the club drug MDMA (Baumann et al. 2007; Sandtner et al. 2016).

In assay systems using human transporters expressed in HEK cells, mephedrone and methylone inhibit neurotransmitter uptake and act as substrates at DAT, NET, and SERT (Eshleman et al. 2013; Simmler et al. 2013; Mayer et al. 2016; Pifl et al. 2015),

consistent with findings in synaptosomes. Importantly, voltage-clamp experiments carried out in *Xenopus* oocytes expressing either DAT or SERT reveal that mephedrone induces robust inward sodium currents, whereas the pyrrolidine-containing cathinone MDPV does not (Cameron et al. 2013; Solis 2017). Other studies show that monensin treatment markedly enhances transporter-mediated release of [^{3}H]MPP$^+$ evoked by mephedrone in HEK cells (Mayer et al. 2016). The unpublished data depicted in Fig. 3 show that DAT-mediated efflux of [^{3}H]MPP$^+$ produced by mephedrone is significantly augmented in the presence of monensin, whereas the modest effects of MDPV are unaffected. Taken together, the results from studies using rat and human transporters agree that ring-substituted cathinones like mephedrone and methylone are transporter substrates capable of inducing transmitter release via DAT, NET, and SERT.

Older studies examining the pharmacology of amphetamine analogs demonstrated that adding bulky substituents to the phenyl ring enhances potency at SERT relative to DAT. For example, the 3-trifluoromethyl analog of amphetamine, norfenfluramine, has much greater potency as a SERT substrate when compared to amphetamine itself (Rothman et al. 2003a). In a similar manner, Cozzi et al. demonstrated that 4-trifluoromethyl-*N*-methylcathinone is a much more potent substrate at SERT than DAT, whereas the parent compound methcathinone displays the opposite selectivity (Cozzi et al. 2013). It is noteworthy that Cozzi et al. also showed that methcathinone is a powerful locomotor stimulant in rats, whereas its 4-trifluoromethyl analog is not, suggesting an increase in potency at SERT is inhibitory to motor stimulant actions. Bonano et al. carried out the first detailed SAR studies to investigate the role of *para*-position (i.e., 4-position) ring substitution on the biological activity of methcathinone analogs (Bonano et al. 2015; Sakloth et al. 2015). In their work, substituents of increasing size (i.e., increasing steric volume) were added to the 4-position of methcathinone, and substrate activity was examined

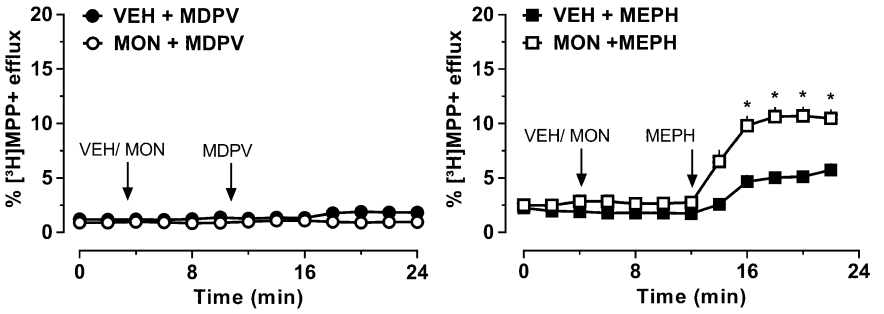

Fig. 3 Effects of monensin on [^{3}H]MPP$^+$ efflux induced by MDPV or mephedrone in HEK cells expressing human DAT. Vehicle (VEH, physiological buffer) or 10 μM monensin (MON) was added to the perfusion solution at 4 min, whereas 10 μM MDVP or 10 μM mephedrone (MEPH) was added at 12 min. Data are mean ± SD for $N = 3$ experiments performed in triplicate. Note than MON has no effect on efflux produced by MDPV (left panel) but markedly enhances efflux produced by the substrate MEPH (right panel). * denotes significance with respect to VEH + MEPH group, $P < 0.05$

at DAT and SERT in rat brain synaptosomes. The findings summarized in Table 2 reveal that increasing steric bulk at the 4-position enhances potency at SERT relative to DAT, thereby decreasing the DAT/SERT ratio of the compounds. The same compounds were tested in the rat intracranial self-stimulation (ICSS) paradigm which can identify abuse-related and abuse-limiting effects of drugs (Negus and Miller 2014). It was found that increasing steric bulk on the 4-position is associated with reduced abuse-related effects, and enhanced abuse-limiting effects, of the analogs. A high positive correlation was shown between DAT/SERT ratio and abuse potential of the analogs. The summed findings predict that drugs with a high DAT/SERT ratio will have high abuse potential, whereas those with lower DAT/SERT ratio will have low abuse liability (Negus and Banks 2017).

Investigations carried out in HEK cells transfected with human transporters also showed that adding substituents to the 4-position of cathinone-related compounds increases activity at SERT relative to DAT. Rickli et al. showed that 4-methyl, 4-ethyl, and 4-bromo analogs of methcathinone have enhanced potency to inhibit [^{3}H]neurotransmitter uptake at SERT when compared to methcathinone (Rickli et al. 2015). Eshleman et al. examined transporter-mediated uptake inhibition and [^{3}H]neurotransmitter release for a series of methcathinone analogs and found that 4-chloro and 4-bromo analogs display greater potency at SERT when compared to methcathinone (Eshleman et al. 2017). Molecular modeling studies based on the crystal structure of *Drosophila* DAT provide evidence that a subregion of the substrate-binding pocket of SERT is slightly larger than that of DAT, allowing for accommodation of larger phenyl ring substituents in SERT (Sakloth et al. 2015). More research investigations using molecular docking approaches and

Table 2 Effects of *para*-position (i.e., 4-position) ring substitution on potency to release [^{3}H]MPP+ via DAT and [^{3}H]5-HT via SERT in rat brain synaptosomes

Para group	Drug	Steric volume (cubic A)	DAT release EC_{50} (nM)	SERT release EC_{50} (nM)	DAT/ SERT ratio
H	Methcathinone (MCAT)	150.4	12.5	3,860	309
F	4-Fluoro MCAT (flephedrone)	153.8	83.4	1,290	15.4
Cl	4-Chloro MCAT (clephedrone)	164.4	42.2	144	3.40
CH_3	4-Methyl MCAT (mephedrone)	166.9	49.1	118	2.41
Br	4-Bromo MCAT (brephedrone)	169.9	59.4	60.2	1.01
OCH_3	4-Methoxy MCAT (methedrone)	175.0	506	120	0.24
CF_3	4-Trifluoromethyl (4-TFM MCAT)	178.4	2,700	190	0.07

Data are mean EC_{50} values for $N = 3$ experiments performed in triplicate, adapted from Bonano et al. (2015). DAT/SERT ratio = $(\text{DAT } EC_{50})^{-1}/(\text{SERT } EC_{50})^{-1}$, where higher value indicates greater DAT selectivity.

dynamic simulations are warranted to address the precise underpinnings of transporter selectivity for ring-substituted cathinones (Zdrazil et al. 2016; Seddik et al. 2017).

4.2 Pyrrolidine-Containing Cathinones Are Transporter Inhibitors

Investigations from the 1990s revealed that pyrovalerone, a structural analog of MDPV (see Fig. 1), is a potent dopamine uptake blocker which produces psychomotor stimulant effects when administered to rodents (Vaugeois et al. 1993; Héron et al. 1994). A seminal study by Meltzer et al. (2006) examined the monoamine transporter activities for several pyrovalerone analogs and showed these agents are potent inhibitors of DAT and NET, with minimal activity at SERT. Importantly, the study of Meltzer and colleagues did not address whether pyrovalerone analogs might act as transporter substrates, and no assessment of MDPV pharmacology was included. To this end, we examined the in vitro transporter activity of MDPV in rat brain synaptosomes and showed the drug displays potent uptake inhibition at DAT and NET, with much weaker activity at SERT (Baumann et al. 2013). The in vitro results with MDPV are consistent with prior data of Meltzer et al. showing that pyrovalerone analogs are potent blockers of DAT and NET. When compared to the prototypical uptake inhibitor cocaine, MDPV is 50-fold more potent as an inhibitor at DAT, tenfold more potent at NET, and tenfold less potent at SERT (see Table 3).

In assays using HEK cells expressing human transporters, Eshleman et al. (2013) and Simmler et al. (2013) confirmed that MDPV is a potent blocker at DAT and NET, but not SERT, and the drug does not evoke transporter-mediated release. These same

Table 3 Effect of α-carbon alkyl chain length on potency to inhibit uptake of [^{3}H]neurotransmitters via DAT, NET, and SERT in rat brain synaptosomes

Alpha-carbon Chain length	Drug	DAT uptake inhibition IC_{50} (nM)	NET uptake inhibition IC_{50} (nM)	SERT uptake inhibition IC_{50} (nM)	DAT/SERT ratio
	Cocaine	211	292	313	1.5
3C (Propyl)	MDPV	4.1	25.9	3,305	806
4C (Butyl)	α-PHP	11.4	26.3	>10,000	>877
3C (Propyl)	α-PVP	12.8	14.2	>10,000	>781
2C (Ethyl)	α-PBP	63.3	91.5	>10,000	>159
1C (Methyl)	α-PPP	196	445	>10,000	>51

Data are mean IC_{50} values for $N = 3$ experiments, adapted from Marusich et al. (2014), except for α-PHP data which are unpublished. DAT/SERT ratio = $(\text{DAT } IC_{50})^{-1}/(\text{SERT } IC_{50})^{-1}$, where higher value indicates greater DAT selectivity

investigators examined the potency of MDPV at various G protein-coupled receptor subtypes and found no significant affinity of the drug for non-transporter sites of action (Eshleman et al. 2013; Simmler et al. 2013). One recent study using single-cell amperometric methods reported that low concentrations of MDPV cause reverse transport of dopamine via DAT, suggestive of substrate activity (Shekar et al. 2017). However, as noted previously, MDPV does not induce transporter-associated inward currents in DAT-expressing *Xenopus* oocytes (Cameron et al. 2013; Solis 2017), and effects of the drug in HEK cells are not affected by monensin treatment. Thus, findings from a variety of different assay methods in native tissues and transporter-expressing cells indicate that MDPV is a potent blocker at DAT and NET, with minimal substrate activity.

Kolanos et al. (2013) performed an SAR study which "deconstructed" the MDPV molecule piece-by-piece to determine which structural features govern its ability to inhibit [^{3}H]dopamine uptake in cells transfected with human DAT. It was found that the bulky pyrrolidine ring and the flexible α-carbon chain were critical attributes for potent uptake inhibition at DAT, whereas the 3,4-methylenedioxy ring moiety was of minor consequence. Marusich et al. (2014) followed up this study and confirmed that removing the 3,4-methylenedioxy ring substituent of MDPV, to form α-pyrrolidinovalerophenone (α-PVP), has little influence on potency to inhibit DAT or NET in rat brain synaptosomes. These investigators also examined the effects of altering α-carbon chain length on potency to inhibit [^{3}H]neurotransmitter uptake at DAT, NET, and SERT. The data summarized in Table 3 show that decreasing the α-carbon chain length of α-PVP to form α-pyrrolidinobutiophenone (α-PBP) and α-pyrrolidinopropiophenone (α-PPP) produces a stepwise reduction in potency to inhibit uptake at DAT and NET. Nevertheless, all of the pyrrolidine-containing compounds maintain high selectivity at DAT and NET over SERT, with DAT/SERT ratios ranging from 50 to 800. It is noteworthy that α-PPP, the weakest compound tested, is similar in potency to cocaine at DAT and NET. Increasing the α-carbon chain length to a butyl group to form α-pyrrolidinohexiophenone (α-PHP) engenders similar potency to α-PVP. Indeed, analogs of α-PVP with large bulky groups attached at the α-carbon position (e.g., cyclohexyl) retain high potency as blockers of DAT and NET (Kolanos et al. 2015a).

The formulation of MDPV available in the recreational drug marketplace is a racemic mixture of (*S*) and (*R*) stereoisomers. Meltzer et al. (2006) showed that (*S*)-pyrovalerone is much more potent as a blocker at DAT and NET when compared to (*R*)-pyrovalerone, indicating MDPV stereoisomers might exhibit a similar degree of transporter selectivity. Kolanos et al. (2015b) reported the stereoselective synthesis of MDPV enantiomers using (*S*)- and (*R*)-norvaline as starting materials, whereas Suzuki et al. (2015) resolved MDPV enantiomers from the racemic mixture. In the study of Kolanos et al., (*S*)-MDPV was 100 times more potent at inhibiting DAT when compared to (*R*)-MDPV. Therefore, similar to the findings reported for pyrovalerone, the biological activity of racemic MDPV resides primarily with the (*S*)-isomer. In agreement with the in vitro transporter results, (*S*)-MDPV is much more potent than (*R*)-MDPV in eliciting locomotor stimulant and reinforcing effects in both rats and mice (Kolanos et al. 2015b; Gannon et al. 2016, 2017).

5 In Vivo Pharmacology

5.1 In Vivo Microdialysis

Drugs acting as inhibitors or substrates at DAT, NET, and SERT increase the extracellular concentrations of dopamine, norepinephrine, and 5-HT in the brain to enhance monoamine signaling (Rothman and Baumann 2003; Howell and Kimmel 2008). We use in vivo methods to simultaneously examine neurochemical and behavioral effects of transporter ligands in rats (Rothman et al. 2005; Baumann et al. 2011). Specifically, in vivo microdialysis is used to collect samples of extracellular fluid (i.e., dialysate samples) from the brains of conscious freely behaving rats. The microdialysis probes are placed into the nucleus accumbens, a brain region implicated in the locomotor stimulant and rewarding effects of abused drugs (Willuhn et al. 2010; Ikemoto and Bonci 2014), and dialysate samples are analyzed for concentrations of dopamine and 5-HT using high-performance liquid chromatography coupled to electrochemical detection. Rats undergoing microdialysis are housed in arenas equipped with photo beams sensitive to locomotor activity in the horizontal plane (i.e., ambulation) and repetitive back-and-forth movements of the head, trunk, and limbs (i.e., stereotypy). Our methods allow for the assessment of relationships between extracellular monoamines and behavior. In previous studies, we found a significant positive correlation between the amount of dialysate dopamine in the nucleus accumbens and the extent of locomotor activation produced by transporter ligands (Zolkowska et al. 2009; Baumann et al. 2011). Furthermore, data reveal that elevations in dialysate 5-HT alone are not sufficient to produce locomotor activation (Cozzi et al. 2013), but elevations in extracellular 5-HT can dampen the motor stimulant effects mediated by concurrent elevations in extracellular dopamine (Rothman et al. 2005; Baumann et al. 2011).

Kehr et al. first reported that subcutaneous (s.c.) administration of mephedrone to rats evokes elevations in extracellular dopamine and 5-HT in the nucleus accumbens (Kehr et al. 2011), and other research groups confirmed these findings in rats receiving either s.c. or intraperitoneal (i.p.) mephedrone injections (Wright et al. 2012; Shortall et al. 2016; Suyama et al. 2016). We found that intravenous (i.v.) administration of mephedrone or methylone produces dose-related increases in extracellular dopamine and 5-HT in rat nucleus accumbens, with mephedrone being slightly more potent than methylone (Baumann et al. 2012; Mayer et al. 2016; Elmore et al. 2017). Rats repeatedly exposed to mephedrone during adolescence and re-exposed later in life show a potentiation of dopamine and 5-HT release in the nucleus accumbens and prefrontal cortex, indicating the development of neurochemical sensitization (Kaminska et al. 2018). Interestingly, most microdialysis studies testing the acute effects of mephedrone and methylone have found the magnitude of increase in dialysate 5-HT exceeds the accompanying increase in dialysate dopamine. This profile of in vivo neurochemical effects produced by mephedrone and methylone is consistent with the nonselective substrate activity of these drugs and mimics the known neurochemical effects of MDMA (Baumann et al. 2008, 2012; Kehr et al. 2011). We first reported that i.v. administration of MDPV to

rats produces dose-related increases in extracellular dopamine but not 5-HT, and MDPV is tenfold more potent than cocaine in this regard (Baumann et al. 2013). The ability of MDPV to increase extracellular dopamine has been confirmed in rats and mice (Johnson et al. 2018; Wojcieszak et al. 2018). The rise in extracellular dopamine produced by MDPV is fully consistent with the potent inhibition of dopamine uptake produced by the drug in vitro.

Microdialysis methods have been used to elucidate mechanisms underlying the elevations in extracellular neurotransmitters produced by transporter ligands in vivo (Nomikos et al. 1990; Chen and Reith 1994; Gundlah et al. 1997). In particular, reserpine has been used as a pharmacological tool to distinguish between the effects of transporter blockers versus substrates. Reserpine is a naturally occurring indole alkaloid that induces long-lasting depletion of monoamine neurotransmitters from synaptic vesicles in the brain and periphery (Arbuthnott et al. 1990). A number of microdialysis investigations have shown that reserpine pretreatment blocks cocaine-induced increases in extracellular dopamine and norepinephrine in rat brain, without affecting amphetamine-induced neurotransmitter increases (Butcher et al. 1988; Callaway et al. 1989; Florin et al. 1995). These data demonstrate that transporter blockers like cocaine increase dialysate neurotransmitter concentrations via a vesicular pool linked to exocytosis, while transporter substrates like amphetamine can increase dialysate neurotransmitter concentrations from a non-vesicular pool. We recently carried out a microdialysis investigation comparing the effects of MDPV and mephedrone in rats pretreated with reserpine. Male Sprague-Dawley rats were pretreated with 5 mg/kg s.c. reserpine 24 h before being subjected to microdialysis testing. The unpublished data in Fig. 4 show that MDPV-induced dopamine elevations are significantly blunted by reserpine pretreatment, but the effects of mephedrone are not altered. The findings from reserpinized rats indicate that MDPV acts as a transporter blocker in vivo, with dopamine responses dependent upon ongoing exocytotic transmitter release. By contrast, mephedrone acts as a transporter substrate in vivo that can release dopamine from a non-vesicular pool. Future microdialysis studies should address the in vivo mechanisms of action for ring-substituted and pyrrolidine-containing cathinones.

5.2 Locomotor Activation

Studies from the 1980s revealed that cathinone and methcathinone display powerful locomotor stimulant effects in mice and rats (Kalix 1980; Glennon et al. 1987). Accordingly, most of the synthetic cathinones found in the recreational drug marketplace are reported to stimulate locomotor activity when administered to rats (Baumann et al. 2012; Huang et al. 2012; Aarde et al. 2013a; Shortall et al. 2013) or mice (López-Arnau et al. 2012; Marusich et al. 2012; Fantegrossi et al. 2013; Gatch et al. 2013). In one of the first studies to examine the behavioral effects of bath salts cathinones, Marusich et al. showed that mephedrone, methylone, and MDPV produce dose-dependent increases in ambulation in mice, and MDPV is somewhat more potent in this regard (Marusich et al. 2012). We found that MDPV is about

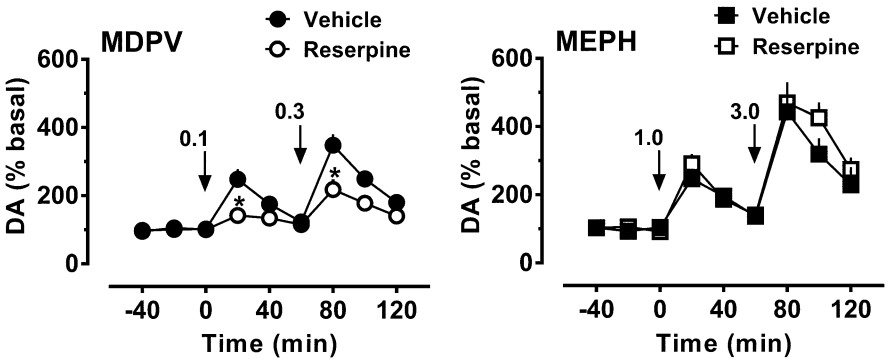

Fig. 4 Effects of reserpine pretreatment on extracellular dopamine responses produced by MDPV or mephedrone in rats undergoing microdialysis in nucleus accumbens. Rats received 5 mg/kg i.p. reserpine 24 h prior to microdialysis testing. Rats received i.v. injections of MDPV or mephedrone (MEPH) at time zero and 60 min later. Data are mean ± SEM for $N = 6$ rats/group, depicted as % basal calculated from three preinjection samples. Basal dopamine concentrations in control and reserpinized rats were 3.98 ± 0.88 and 1.44 ± 0.22 pg/μL, respectively. Note that reserpine reduces dialysate dopamine responses produced by the blocker MDPV (left panel), without altering effects of the substrate MEPH (right panel). * denotes significant difference from vehicle-treated group, $P < 0.05$

tenfold more potent than cocaine as a locomotor stimulant in rats, and MDPV is also more efficacious than cocaine, stimulating an overall greater magnitude of motor activation (Baumann et al. 2013). When MDPV and other synthetic cathinones are administered across a broad range of doses, the dose-response relationship for ambulation is an inverted U-shaped function (Aarde et al. 2013a; Gatch et al. 2013); the reduction in forward locomotion at higher drug doses is due to the emergence of focused stereotypies, such as in-place perseverative sniffing and head bobbing, as dose increases. Recent studies demonstrate that MDPV and its unsubstituted phenyl ring analogs α-PVP, α-PBP, and α-PPP induce dose-related stimulation of locomotor activity in mice, and in vivo potency is correlated with in vitro DAT activity (Marusich et al. 2014). Specifically, as the α-carbon alkyl chain length decreases across the series of compounds, potency to induce motor activation and inhibit uptake at DAT decrease in parallel (see Table 3). In mice, the locomotor stimulant effects of MDPV and α-PVP were significantly blunted by pretreatment with antagonists for either D_1 or D_2 dopamine receptors (Kaizaki et al. 2014; Marusich et al. 2014). Taken together with the microdialysis data, the available evidence indicates that MDPV, and perhaps other synthetic cathinones, elevate extracellular dopamine in critical brain circuits via DAT inhibition, and subsequent activation of D_1 and D_2 receptors by endogenous dopamine underlies locomotor stimulant effects of the drugs.

The role of extracellular 5-HT in modulating the dopaminergic effects of synthetic cathinones is a topic of great interest. As mentioned already, most ring-substituted cathinones act as nonselective transporter substrates, which can increase extracellular concentrations of both dopamine and 5-HT. A recent investigation

compared the neurochemical and locomotor effects of MDPV and methylone in rats to examine potential interactions between dopaminergic and serotonergic effects of these drugs (Schindler et al. 2016). It was found that i.v. doses of 0.3 mg/kg MDPV and 3.0 mg/kg methylone produce nearly identical threefold elevations in extracellular dopamine, whereas only methylone produces a dramatic tenfold elevation in extracellular 5-HT. At these same doses, MDPV elicits a much greater stimulation of ambulation and stereotypy when compared to methylone. The data comparing MDPV and methylone are reminiscent of the data comparing methcathinone and its 4-trifluoromethyl analog; methcathinone is a potent DAT substrate with robust motor stimulant effects, but 4-trifluoromethyl-*N*-methcathinone (4-TFM MCAT) is a SERT-preferring substrate with minimal stimulant effects (Cozzi et al. 2013). One interpretation of these findings is that elevations in extracellular 5-HT tend to reduce locomotor stimulant effects mediated by extracellular dopamine. Indeed, substantial evidence indicates that high-affinity 5-HT_{2C} receptor sites in the brain provide a strong inhibitory influence over dopamine-mediated behavioral effects of cocaine and other psychomotor stimulant drugs of abuse (Devroye et al. 2013; Howell and Cunningham 2015). Future studies are warranted to examine the role of 5-HT receptor subtypes in modulating the motor stimulant effects of new synthetic cathinones as they appear in the recreational drug marketplace.

5.3 Rewarding Effects

The acute rewarding effects of abused drugs are related to their ability to increase extracellular dopamine concentrations in the nucleus accumbens, by acting directly or indirectly with mesocorticolimbic neuronal pathways (Di Chiara and Imperato 1988; Volkow and Morales 2015). Psychomotor stimulant drugs are capable of directly activating mesolimbic dopamine neurons because they act as DAT blockers or substrates which increase extracellular dopamine concentrations. Despite many differences in complex behaviors between humans and animal species, the self-administration of abused drugs is largely conserved among humans (Henningfield et al. 1991), nonhuman primates (Beveridge et al. 2006), rats (Deroche-Gamonet et al. 2004), and mice (Yan et al. 2014). In the case of synthetic cathinones, animal studies are critical for determining the rewarding effects of these drugs because no controlled laboratory studies in humans have been carried out. Moreover, results from animal studies are needed to assess risk and inform legislative decisions for banning specific drugs as they emerge in the recreational drug marketplace. Two experimental paradigms that have been used to characterize the abuse liability of drugs in rats are drug self-administration and ICSS.

Drug self-administration is often considered the "gold standard" behavioral test for determining the abuse potential of drugs (Watterson and Olive 2017). In the rat drug self-administration paradigm, animals with surgically implanted i.v. catheters are trained to lever-press or nose-poke to obtain i.v. drug injections which are delivered via a computer-controlled infusion pump. The data reviewed thus far predict that DAT/NET-selective drugs like MDPV would engender potent rewarding

effects. Indeed, rats rapidly learn to self-administer MDPV under fixed ratio conditions and maintain high rates of responding during 1 or 2 h self-administration sessions (Aarde et al. 2013a; Watterson et al. 2014; Schindler et al. 2016). When tested in the progressive ratio test, MDPV shows breakpoints similar to methamphetamine and amphetamine, confirming the efficacious reinforcing properties of MDPV. In addition, when access to MDPV is extended to 6 h, MDPV shows escalation of drug intake for doses between 0.1 and 0.5 mg/kg i.v. (Watterson et al. 2014). More recent studies show that MDPV analogs like α-PVP are also readily self-administered, with potency and efficacy comparable to MDPV itself (Aarde et al. 2015; Huskinson et al. 2017; Gannon et al. 2018). Taken together, the self-administration data with pyrrolidine-containing cathinones indicate these compounds are highly rewarding and possess risk for compulsive use.

The neurochemical effects of mephedrone and methylone are similar to the effects of MDMA, the prototypical "entactogen-type" drug of abuse. The term entactogen is used to describe agents like MDMA, which engender feelings of emotional communion, oneness, or relatedness in human users (Aarde and Taffe 2017). The unique subjective effects of MDMA are presumably related to elevations in extracellular 5-HT, which subsequently activates 5-HT receptor subtypes, particularly 5-HT_{2A} sites, in the brain (Liechti and Vollenweider 2001; Farre et al. 2007; Hysek et al. 2012). The serotonergic activity of ring-substituted cathinones might be predicted to reduce their abuse liability since preclinical evidence supports a role for 5-HT in dampening reinforcing effects of stimulant drugs (Wee et al. 2005; Wee and Woolverton 2006). However, a number of studies in rats demonstrate that mephedrone and methylone are readily self-administered and maintain high rates of drug-appropriate responding (Hadlock et al. 2011; Watterson et al. 2012; Aarde et al. 2013b; Motbey et al. 2013; Schindler et al. 2016). In particular, the pattern of mephedrone self-administration under fixed ratio schedules seems closer to highly dependence-producing drugs like methamphetamine rather than MDMA, though mephedrone is about tenfold less potent than methamphetamine (Aarde et al. 2013; Motbey et al. 2013). Taffe and colleagues directly compared self-administration behavior for various entactogens and found mephedrone-trained rats show much higher levels of responding than either methylone- or MDMA-trained rats (Creehan et al. 2015; Vandewater et al. 2015), pointing to higher abuse liability for mephedrone. Importantly, when the duration of self-administration sessions is extended from 2 to 6 h, mephedrone, methylone, and MDMA all display escalation of drug intake consistent with compulsive use (Vandewater et al. 2015). Collectively, the self-administration data published thus far indicate that mephedrone and methylone display substantial abuse liability. These findings suggest that rewarding effects of synthetic cathinones are complex and may involve neurotransmitter systems in addition to dopamine and 5-HT (e.g., norepinephrine). More studies are needed to clarify the role of specific neurotransmitter systems, and their receptor subtypes, in modulating rewarding effects of ring-synthetic cathinones.

ICSS is a technique that can be used to characterize the rewarding effects of drugs. ICSS involves the electrical stimulation of the medial forebrain bundle, a collection of nerve fibers including axons of ascending dopaminergic projections from the ventral

tegmental area to the nucleus accumbens, which sustains reward in different species (Negus and Miller 2014). One important feature of the ICSS paradigm is the ability to discern abuse-related and abuse-limiting effects of drugs. For example, prior ICSS studies show that most monoamine transporter ligands induce facilitation of ICSS responding, which is an abuse-related effect mediated by extracellular dopamine. By contrast, the SERT-selective substrate fenfluramine induces suppression of ICSS responding, an abuse-limiting effect (Bauer et al. 2013). Bonano et al. compared ICSS responses following administration of MDPV, mephedrone, or methylone and showed all three compounds facilitate ICSS responding, consistent with abuse-related and rewarding effects of the drugs. However, there were important differences across the compounds tested. MDPV engenders robust ICSS facilitation which is comparable to the effects of methcathinone and has a long duration of action. Methylone showed comparable effects to MDPV in terms of ICSS facilitation and duration of action, but it is tenfold less potent than MDPV. Surprisingly, mephedrone was the weakest of the compounds tested to facilitate ICCS responding (Bonano et al. 2014). The finding that methylone induces greater facilitation of ICSS responding when compared to mephedrone contrasts with the self-administration data discussed previously, which clearly show mephedrone is a more potent and efficacious reinforcer. It seems possible that drug self-administration and ICSS responding may involve distinct yet overlapping circuitries which can yield disparate results.

Bonano et al. carried out a study to examine the SAR of ring-substituted cathinones with different substituents at the *para*-position of the phenyl ring (Bonano et al. 2015). By modifying the same position of the methcathinone scaffold, it was possible to obtain transporter substrates ranging from 300-fold selective for DAT over SERT (e.g., methcathinone) to 20-fold selective for SERT over DAT (e.g., 4-trifluoromethyl-*N*-methcathinone). As discussed above, abuse-related effects of the compounds were positively correlated with DAT/SERT ratio, and highly DAT-selective analogs strongly facilitated ICSS responding. Nonselective analogs produced mild and variable facilitation of ICSS, and highly SERT-selective analogs strongly depressed ICSS responding.

6 Summary

The research investigations reviewed in this chapter show that synthetic cathinones interact with monoamine transporter proteins as either blockers or substrates. Pyrrolidine-containing cathinones like MDPV and α-PVP are potent uptake blockers at DAT and NET, with much less potent effects at SERT. The bulky pyrrolidine ring and α-carbon alkyl chain are critical determinants of DAT/NET activity, and shorter α-carbon chain length is associated with decreased potency. Most evidence indicates that pyrrolidine-containing cathinones are devoid of substrate activity, perhaps because they are sterically too large to fit through the transporter permeation pore. Ring-substituted cathinones like mephedrone and methylone are nonselective transporter substrates, which induce non-exocytotic release of dopamine, norepinephrine, and 5-HT by reverse transport. Due to their substrate activity, mephedrone and

methylone are capable of inducing transporter-associated ionic currents. Ring-substituted cathinones with bulky *para* substituents have enhanced activity at SERT, and DAT/SERT selectivity decreases as substituent size increases.

Regardless of whether synthetic cathinones act as blockers or substrates, they all increase extracellular concentrations of monoamines in brain reward pathways. Drug-induced elevations in extracellular dopamine in the nucleus accumbens appear to underlie locomotor and rewarding effects in vivo, consistent with the effects of other stimulant drugs. Importantly, the precise molecular mechanism of drug action is less important than overall selectivity across DAT, NET, and SERT. In general, synthetic cathinones with high DAT selectivity are potent and efficacious reinforcers, whereas those with high SERT selectivity are less reinforcing. However, there are many caveats to this simplistic view. A number of synthetic cathinones with mixed DAT/SERT activity display greater abuse liability than MDMA in animal models. The in vivo data with mephedrone are most intriguing, since this compound acts as an efficacious reinforcer in self-administration assays but is weak in its ability to facilitate ICSS responding. Despite the increasing knowledge about the neuropharmacology of synthetic cathinones, many questions remain unanswered, including the poorly understood role of non-transporter sites of action, drug pharmacokinetics, and drug metabolism. More research is warranted to examine the biological effects of synthetic cathinones in rodent models, especially investigations aimed at determining the mechanisms underlying motor stimulant and rewarding effects of the drugs.

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References

Aarde SM, Huang PK, Creehan KM, Dickerson TJ, Taffe MA (2013a) The novel recreational drug 3,4-methylenedioxypyrovalerone (MDPV) is a potent psychomotor stimulant: self-administration and locomotor activity in rats. Neuropharmacology 71:130–140

Aarde SM, Angrish D, Barlow DJ, Wright MJ Jr, Vandewater SA, Creehan KM et al (2013b) Mephedrone (4-methylmethcathinone) supports intravenous self-administration in Sprague-Dawley and Wistar rats. Addict Biol 18(5):786–799

Aarde SM, Creehan KM, Vandewater SA, Dickerson TJ, Taffe MA (2015) In vivo potency and efficacy of the novel cathinone α-pyrrolidinopentiophenone and 3,4-methylenedioxypyrovalerone: self-administration and locomotor stimulation in male rats. Psychopharmacology (Berl) 232 (16):3045–3055

Adams SV, DeFelice LJ (2003) Ionic currents in the human serotonin transporter reveal inconsistencies in the alternating access hypothesis. Biophys J 85(3):1548–1559

Al-Hebshi NN, Skaugh N (2005) Khat (Catha edulis)- an updated review. Addict Biol 10 (4):299–307

Alexander SP, Kelly E, Marrion NV, Peters JA, Faccenda E, Harding SD (2017) The concise guide to pharmacology 2017/18: transporters. Br J Pharmacol 174(Suppl 1):S360–S446

Arbuthnott GW, Fairbrother IS, Butcher SP (1990) Dopamine release and metabolism in the rat striatum: an analysis by 'in vivo' brain microdialysis. Pharmacol Ther 48(3):281–293

Axelrod J, Whitby LG, Hertting G (1961) Effect of psychotropic drugs on the uptake of H^3-norepinephrine by tissues. Science 133(3450):383–384

Banks ML, Worst TJ, Rusyniak DE, Sprague JE (2014) Synthetic cathinones ("bath salts"). J Emerg Med 46(5):632–642

Bauer CT, Banks ML, Blough BE, Negus SS (2013) Use of intracranial self-stimulation to evaluate abuse-related and abuse-limiting effects of monoamine releasers in rats. Br J Pharmacol 168 (4):850–862

Baumann MH, Wang X, Rothman RB (2007) 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. Psychopharmacology (Berl) 189 (4):407–424

Baumann MH, Clark RD, Rothman RB (2008) Locomotor stimulation produced by 3,4-methylenedioxymethamphetamine (MDMA) is correlated with dialysate levels of serotonin and dopamine in rat brain. Pharmacol Biochem Behav 90(2):208–217

Baumann MH, Clark RD, Woolverton WL, Wee S, Blough BE, Rothman RB (2011) In vivo effects of amphetamine analogs reveal evidence for serotonergic inhibition of mesolimbic dopamine transmission in the rat. J Pharmacol Exp Ther 337(1):218–225

Baumann MH, Ayestas MA Jr, Partilla JS, Sink JR, Shulgin AT, Daley PF et al (2012) The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. Neuropsychopharmacology 37(5):1192–1203

Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF, Holy M (2013) Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive "bath salts" products. Neuropsychopharmacology 38(4):552–562

Baumann MH (2014) Awash in a sea of 'bath salts': implications for biomedical research and public health. Addiction 109(10):157–159

Baumann MH, Solis E Jr, Watterson LR, Marusich JA, Fantegrossi WE, Wiley JL (2014a) Bath salts, spice, and related designer drugs: the science behind the headlines. J Neurosci 34 (46):15150–15158

Baumann MH, Bulling S, Benaderet TS, Saha K, Avestas MA, Partilla JS et al (2014b) Evidence for a role of transporter-mediated currents in the depletion of brain serotonin induced by serotonin transporter substrates. Neuropsychopharmacology 39(6):1355–1365

Beveridge TJ, Smith HR, Daunais JB, Nader MA, Porrino LJ (2006) Chronic cocaine self-administration is associated with altered functional activity in the temporal lobes of non human primates. Eur J Neurosci 23(11):3109–3118

Bonano JS, Glennon RA, De Felice LJ, Banks ML, Negus SS (2014) Abuse-related and abuse-limiting effects of methcathinone and the synthetic "bath salts" cathinone analogs methylenedioxypyrovalerone (MDPV), methylone and mephedrone on intracranial self-stimulation in rats. Psychopharmacology (Berl) 231(1):199–207

Bonano JS, Banks ML, Kolanos R, Sakloth F, Barnier ML, Glennon RA et al (2015) Quantitative structure-activity relationship analysis of the pharmacology of para-substituted methcathinone analogues. Br J Pharmacol 172(10):2433–2444

Bönisch H (1986) The role of co-transported sodium in the effect of indirectly acting sympathomimetic amines. Naunyn Schmiedebergs Arch Pharmacol 332(2):135–141

Butcher SP, Fairbrother IS, Kelly JS, Arbuthnott GW (1988) Amphetamine-induced dopamine release in the rat striatum: an in vivo microdialysis study. J Neurochem 50(2):346–355

Callaway CW, Kuczenski R, Segal DS (1989) Reserpine enhances amphetamine stereotypies without increasing amphetamine-induced changes in striatal dialysate dopamine. Brain Res 505(1):83–90

Cameron KN, Kolanos R, Solis E Jr, Glennon RA, Da Felice LF (2013) Bath salts components mephedrone and methylenedioxypyrovalerone (MDPV) act synergistically at the human dopamine transporter. Br J Pharmacol 168(7):1750–1757

Centers for Disease Control and Prevention (CDC) (2011) Emergency department visits after use of a drug sold as "bath salts" – Michigan, November 13, 2010-March 31, 2011. MMWR Morb Mort Wkly Rep 60(19):624–627

Cercato C, Roizenblatt VA, Leança CC, Segal A, Lopes Filho AP, Mancini MC et al (2009) A randomized double-blind placebo-controlled study of the long-term efficacy and safety of diethylpropion in the treatment of obese subjects. Int J Obes (Lond) 33(8):857–865

Chen NH, Reith ME (1994) Effects of locally applied cocaine, lidocaine, and various uptake blockers on monoamine transmission in the ventral tegmental area of freely moving rats: a microdialysis study on monoamine interrelationships. J Neurochem 63(5):1701–1713

Cozzi NV, Sievert MK, Shulgin AT, Jacob P 3rd, Ruoho AE (1999) Inhibition of plasma membrane monoamine transporters by beta-ketoamphetamines. Eur J Pharmacol 281(1):63–69

Cozzi NV, Brandt SD, Daley PF, Partilla JS, Rothman RB, Tulzer A et al (2013) Pharmacological examination of trifluoromethyl ring-substituted methcathinone analogs. Eur J Pharmacol 699 (1–3):180–187

Creehan KM, Vandewater SA, RAffe MA (2015) Intravenous self-administration of mephedrone, methylone and MDMA in female rats. Neuropharmacology 92:90–97

De Felice LJ, Glennon RA, Negus SS (2014) Synthetic cathinones: chemical phylogeny, physiology, and neuropharmacology. Life Sci 97(1):20–26

Degenhardt L, Baxter AJ, Lee YY, Hall W, Sara GE, Johns N et al (2014) The global epidemiology and burden of psychostimulant dependence: findings from the Global Burden of Disease Study 2010. Drug Alcohol Depend 137:36–47

Deroche-Gamonet V, Belin D, Piazza PV (2004) Evidence for addiction-like behavior in the rat. Science 305(5686):1014–1017

Devroye C, Filip M, Przegalinski E, McCreary AC, Spampinato U (2013) Serotonin2C receptors and drug addiction: focus on cocaine. Exp Brain Res 230(4):537–545

Dhillon S, Yang LP, Curran MP (2008) Bupropion: a review of its use in the management of major depressive disorder. Drugs 68(5):653–689

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85 (14):5274–5278

Drug Enforcement Administration (DEA), Department of Justice (2013) Establishment of drug codes for 26 substances. Final rule. Fed Regist 78(3):664–666

Dwoskin LP, Rauhut AS, King-Paspisil KA, Bardo MT (2006) Review of the pharmacology and clinical profile of bupropion, an antidepressant and tobacco use cessation agent. CNS Drug Rev 12(3–4):178–207

Elmore JS, Dillon-Carter O, Partilla JS, Ellefsen KN, Concheiro M, Suzuki M et al (2017) Pharmacokinetic profiles and pharmacodynamic effects for methylone and its metabolites in rats. Neuropsychopharmacology 42(3):649–660

Emerson TS, Cisek JE (1993) Methcathinone: a Russian designer amphetamine infiltrates the rural midwest. Ann Emerg Med 22(12):1897–1903

Engidawork E (2017) Pharmacological and toxicological effects of Catha edulis F. (Khat). Phytother Res 31(7):1019–1028

Eshleman AJ, Henningsen RA, Neve KA, Janowsky A (1994) Release of dopamine via the human transporter. Mol Pharmacol 45(2):312–316

Eshleman AJ, Wolfrum KM, Hatfield MG, Johnson RA, Murphy KV, Janowsky A (2013) Substituted methcathinones differ in transporter and receptor interactions. Biochem Pharmacol 85(12):1803–1815

Eshleman AJ, Wolfrum KM, Reed JF, Kim SO, Swanson T, Johnson RA (2017) Structure-activity relationships of substituted cathinones, with transporter binding, uptake, and release. J Pharmacol Exp Ther 360(1):33–47

Espana RA, Jones SR (2013) Presynaptic dopamine modulation by stimulant self-administration. Front Biosci (Schol Ed) 5:261–276

Fantegrossi WE, Gannon BM, Zimmerman SM, Rice KC (2013) In vivo effects of abused 'bath salt' constituent 3,4-methylenedioxypyrovalerone (MDPV) in mice: drug discrimination, thermoregulation, and locomotor activity. Neuropsychopharmacology 38(4):563–573

Faraone SV (2018) The pharmacology of amphetamine and methylphenidate: relevance to the neurobiology of attention-deficit/hyperactivity disorder and other psychiatric comorbidities. Neurosci Biobehav Rev 87:255–270

Farre M, Abanades S, Roset PN, Peiro AM, Torrens M, O'Mathuna B et al (2007) Pharmacological interaction between 3,4-methylenedioxymethamphetamine (ecstasy) and paroxetine: pharmacological effects and pharmacokinetics. J Pharmacol Exp Ther 323(3):954–962

Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR (2007) New insights into the mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol 47:681–698

Florin SM, Kuczenski R, Segal DS (1995) Effects of reserpine on extracellular caudate dopamine and hippocampus norepinephrine responses to amphetamine and cocaine: mechanistic and behavioral considerations. J Pharmacol Exp Ther 274(1):231–241

Gannon BM, Williamson A, Suzuki M, Rice KC, Fantegrossi WE (2016) Stereoselective effects of abused "bath salt" constituent 3,4-methylenedioxypyrovalerone in mice: drug discrimination, locomotor activity, and thermoregulation. J Pharmacol Exp Ther 356(3):615–623

Gannon BM, Rice KC, Collins GT (2017) Reinforcing effects of abused 'bath salts' constituents 3,4-methylenedioxypyrovalerone and α-pyrrolidinopentiophenone and their enantiomers. Behav Pharmacol 28(7):578–581

Gannon BM, Galindo KI, Mesmin MP, Sulima A, Rice KC, Collins GT (2018) Relative reinforcing effects of second-generation synthetic cathinones: acquisition of self-administration and fixed-ratio dose-response curves in rats. Neuropharmacology 134(Pt A):28–35

Gatch MB, Taylor CM, Forster MJ (2013) Locomotor stimulant and discriminative stimulus effects of 'bath salt' cathinones. Behav Pharmacol 24(5–6):437–447

Giros B, el Mestikawy S, Bertrand L, Caron MG (1991) Cloning and functional characterization of a cocaine-sensitive dopamine transporter. FEBS Lett 295(1–3):149–154

Glennon RA, Yousif M, Naiman N, Kalix P (1987) Methcathinone: a new and potent amphetamine-like agent. Pharmacol Biochem Behav 26(3):547–551

Glennon RA, Dukat M (2017) Structure-activity relationships of synthetic cathinones. Curr Top Behav Neurosci 32:19–47

Goldberg J, Gardos G, Cole JO (1973) A controlled evaluation of pyrovalerone in chronically fatigued volunteers. Int Pharmacopsychiatry 8(1):60–69

Goldstone MS (1993) 'Cat': methcathinone – a new drug of abuse. JAMA 269(19):2508

Grohol J (2017) Top 25 psychiatric medications for 2016. https://psychcentral.com/blog/top-25-psychiatric-medications-for-2016. Accessed 8 Sep 2018

Gundlah C, Martin KF, Heal DJ, Auerback SB (1997) In vivo criteria to differentiate monoamine reuptake inhibitors from releasing agents: sibutramine is a reuptake inhibitor. J Pharmacol Exp Ther 283(2):581–591

Hadlock GV, Webb KM, McFadden LM, Chu PW, Ellis JD, Allen SC et al (2011) 4-Methylmethcathinone (mephedrone): neuropharmacological effects of a designed stimulant of abuse. J Pharmacol Exp Ther 339(2):530–536

Heikkila RE, Orlansky H, Cogen G (1975) Studies on the distinction between uptake inhibition and release of (3H)dopamine in rat brain tissue slices. Biochem Pharmacol 24(8):847–852

Henningfield JE, Cohen C, Heishman SJ (1991) Drug self-administration methods in abuse liability evaluation. Br J Addict 86(12):1571–1577

Héron C, Costentin J, Bonnet JJ (1994) Evidence that pure uptake inhibitors including cocaine interact slowly with the dopamine neuronal carrier. Eur J Pharmacol 264(3):391–398

Hilber B, Scholze P, Dorostkar MM, Sandtner W, Holy M, Boehm S et al (2005) Serotonin-transporter mediated effux: a pharmacological analysis of amphetamines and non-amphetamines. Neuropharmacology 49(6):811–819

Howell LL, Kimmel HL (2008) Monoamine transporters and psychostimulant addiction. Biochem Pharmacol 75(1):196–217

Howell LL, Cunningham KA (2015) Serotonin 5-HT2 receptor interactions with dopamine function: implications for therapeutics in cocaine use disorder. Pharmacol Rev 67(1):176–197

Huang PK, Aarde SM, Angrish D, Houseknecht KL, Dickerson TJ, Taffe MA (2012) Contrasting effects of d-methamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxypyrovalerone, and 4-methylmethcathinone on wheel activity in rats. Drug Alcohol Depend 126(1–2):168–175

Huestis MA, Brandt SD, Rana S, Auwärter V, Baumann MH (2017) Impact of novel psychoactive substances on clinical and forensic toxicology and global public health. Clin Chem 63 (10):1564–1569

Huskinson SL, Naylor JE, Townsend EA, Rowlett JK, Blough BE, Freeman KB (2017) Self-administration and behavioral economics of second-generation synthetic cathinones in male rats. Psychopharmacology (Berl) 234(4):589–598

Hysek CM, Simmler LD, Nicola VG, Vischer N, Donzelli M, Krahenbuhl S et al (2012) Duloxetine inhibits effects of MDMA ("ecstasy") in vitro and in humans in a randomized placebo-controlled laboratory study. PLoS One 7(5):e36476

Ikemoto S, Bonci A (2014) Neurocircuitry of drug reward. Neuropharmacology 76(Pt B):329–341

Iverson L (2006) Neurotransmitter transporters and their impact on the development of psychopharmacology. Br J Pharmacol 147(Suppl 1):S82–S88

Jardetzky O (1966) Simple allosteric model for membrane pumps. Nature 211(5052):969–970

Johnson AR, Banks ML, Selley DE, Negus SS (2018) Amphetamine maintenance differentially modulates effects of cocaine, methylenedioxypyrovalerone (MDPV), and methamphetamine on intracranial self-stimulation and nucleus accumbens dopamine in rats. Neuropsychopharmacology 43(8):1753–1762

Kaizaki A, Tanaka S, Numazawa S (2014) New recreational drug 1-phenyl-2-(1-pyrrolidinyl)-1-pentanone (alpha-PVP) activates central nervous system via dopaminergic neuron. J Toxicol Sci 39(1):1–6

Kalix P (1980) Hypermotility of the amphetamine type induced by a constituent of khat leaves. Br J Pharmacol 68(1):11–13

Kalix P, Glennon RA (1986) Further evidence for an amphetamine-like mechanism of action of the alkaloid cathinone. Biochem Pharmacol 35(18):3015–3019

Kalix P (1990) Pharmacological properties of the stimulant khat. Pharmacol Ther 48(3):397–416

Kaminski BJ, Griffiths RR (1994) Intravenous self-injection of methcathinone in the baboon. Pharmacol Biochem Behav 27(4):981–983

Kaminska K, Noworyta-Sokolowska K, Górska A, Rzemieniec J, Wnuk A, Wojtas A et al (2018) The effects of exposure to mephedrone during adolescence on brain neurotransmission and neurotoxicity in adult rats. Neurotox Res 34(3):525–537

Karch SB (2015) Cathinone neurotoxicity ("The 3Ms"). Curr Neuropharmacol 13(1):21–25

Kehr J, Ichinose F, Yoshitake S, Goiny M, Sievertsson T, Nyberg F et al (2011) Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats. Br J Pharmacol 164(8):1949–1958

Kilty JE, Lorang D, Amara SG (1991) Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science 254(5031):578–579

Kolanos R, Solis E Jr, Sakloth F, De Felice LJ, Glennon RA (2013) "Deconstruction" of the abused synthetic cathinone methylenedioxypyrovalerone (MDPV) and an examination of effects at the human dopamine transporter. ACS Chem Nerosci 4(12):1524–1529

Kolanos R, Sakloth F, Jain AD, Partilla JS, Baumann MH, Glennon RA (2015a) Structural modification of the designer stimulant α-pyrrolidinovalerophenone (α-PVP) influences potency at dopamine transporters. ACS Chem Nerosci 6(10):1726–1731

Kolanos R, Partilla JS, Baumann MH, Hutsell BA, Banks ML, Negus SS et al (2015b) Stereoselective actions of methylenedioxypyrovalerone (MDPV) to inhibit dopamine and norepinephrine transporters and facilitate intracranial self-stimulation in rats. ACS Chem Nerosci 6(5):771–777

Kristensen AS, Andersen J, Jørgensen TN, Sørensen L, Erikson J, Loland CJ et al (2011) SLC6 neurotransmitter transporters: structure, function and regulation. Pharmacol Rev 63(3):585–640

Liang NY, Rutledge CO (1982) Evidence for carrier-mediated efflux of dopamine from corpus striatum. Biochem Pharmacol 31(15):2479–2484
Liechti ME, Vollenweider FX (2001) Which neuroreceptors mediate the subjective effects of MDMA in humans? A summary of mechanistic studies. Hum Psychopharmacol 16(8):589–598
López-Arnau R, Martinez-Clemente J, Pubill D, Escubedo E, Camarasa J (2012) Comparative neuropharmacology of three psychostimulant cathinone derivatives: butylone, mephedrone and methylone. Br J Pharmacol 167(2):407–420
Madras BK (2017) The growing problem of new psychoactive substances (NPS). Curr Top Behav Neurosci 32:1–18
Mager S, Min C, Henry DJ, Chavkin C, Hoffman BJ, Davidson N (1994) Conducting states of a mammalian serotonin transporter. Neuron 12(4):845–859
Marusich JA, Grant KR, Blough BE, Wiley JL (2012) Effects of synthetic cathinones contained in "bath salts" on motor behavior and a functional observational battery in mice. Neurotoxicology 33(5):1305–1313
Marusich JA, Antonazzo KR, Wiley JL, Blough BE, Partilla JS, Baumann MH (2014) Pharmacology of novel synthetic stimulants structurally related to the "bath salts" constituent 3,4-methylenedioxypyrovalerone (MDPV). Neuropharmacology 87:206–213
Mayer FP, Wimmer L, Dillon-Carter O, Partilla JS, Burchardt NV, Mihovilovic MD (2016) Phase I metabolites of mephedrone display biological activity as substrates at monoamine transporters. Br J Pharmacol 173(17):2657–2668
Mehta NB (1974) Meta chloro substituted-α-butylamino-propiophenones. Burroughs Wellcome Company. North Carolina, USA. US3819706
Meltzer PC, Butler D, Deschamps JR, Madras BK (2006) 1-(4-Methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (pyrovalerone) analogues: a promising class of monoamine uptake inhibitors. J Med Chem 49(4):1420–1432
Mollenhauer HH, Morré DJ, Rowe LD (1990) Alteration of intracellular traffic by monensin; mechanism, specificity and relationship to toxicity. Biochim Biophys Acta 1031(2):225–246
Motbey CP, Clemens KJ, Apetz N, Winstock AR, Ramsey J, Li KM et al (2013) High levels of intravenous mephedrone (4-methylmethcathinone) self-administration in rats: neural consequences and comparison with methamphetamine. J Psychopharmacol 27(9):823–836
Nagai F, Nonaka R, Satoh Hisashi Kamimura K (2007) The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. Eur J Pharmacol 559 (2–3):132–137
Negus SS, Miller LL (2014) Intracranial self-stimulation to evaluate abuse potential of drugs. Pharmacol Rev 66(3):869–917
Negus SS, Banks ML (2017) Decoding the structure of abuse potential for new psychoactive substances: structure-activity relationships for abuse-related effects of 4-substituted methcathinone analogs. Curr Top Behav Neurosci 32:119–131
Nomikos GG, Damsma G, Wenkstern D, Fibiger HC (1990) In vivo characterization of locally applied dopamine uptake inhibitors by striatal microdialysis. Synapse 6(1):106–112
Pacholczyk T, Blakely RD, Amara SG (1991) Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. Nature 350(6316):350–354
Pifl C, Drobny H, Reither H, Hornykiewicz O, Singer EA (1995) Mechanism of the dopamine-releasing actions of amphetamine and cocaine: plasmalemmal dopamine transporter versus vesicular monoamine transporter. Mol Pharmacol 47(2):368–373
Pifl C, Reither H, Hornykiewicz O (2015) The profile of mephedrone on human monoamine transporters differs from 3,4-methylenedioxymethamphetamine primarily by lower potency at the vesicular monoamine transporter. Eur J Pharmacol 755:119–126
Prosser JM, Nelson LS (2012) The toxicology of bath salts: a review of synthetic cathinones. J Med Toxicol 8(1):33–42
Quick MW (2003) Regulating the conducting states of a mammalian serotonin transporter. Neuron 40(3):537–549

Raiteri M, Cerrito F, Cervoni AM, Del Carmine R, Ribera MT, Levi G (1978) Release of dopamine from striatal synaptosomes. Ann Ist Super Sanita 14(1):97–110

Reith ME, Blough BE, Hong WC, Jones KT, Schmitt KC, Baumann MH et al (2015) Behavioral, biological, and chemical perspectives on atypical agents targeting the dopamine transporter. Drug Alcohol Depend 147:1–19

Rickli A, Hoener MD, Liechti ME (2015) Monoamine transporter and receptor interaction profiles of novel psychoactive substances: para-halogenated amphetamines and pyrovalerone cathinones. Eur Neuropsychopharmacol 25(3):365–376

Robertson SD, Matthies HJ, Galli A (2009) A closer look at amphetamine-induced reverse transport and trafficking of the dopamine and norepinephrine transporters. Mol Neurobiol 39(2):73–80

Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI et al (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. Synapse 39(1):32–41

Rothman RB, Baumann MH (2003) Monoamine transporters and psychostimulant drugs. Eur J Pharmacol 479(1–3):23–40

Rothman RB, Clark RD, Partilla JS, Baumann MH (2003a) (+)-Fenfluramine and its major metabolite, (+)-norfenfluramine, are potent substrates for norepinephrine transporters. J Pharmacol Exp Ther 305(3):1191–1199

Rothman RB, Vu N, Partilla JS, Roth BL, Hufeisen SJ, Compton-Toth BA et al (2003b) In vitro characterization of ephedrine-related stereoisomers at biogenic amine transporters and the receptorome reveals selective actions as norepinephrine transporter substrates. J Pharmacol Exp Ther 307(1):138–145

Rothman RB, Blough BE, Woolverton WL, Anderson KG, Negus SS, Mello NK et al (2005) Development of a rationally designed, low abuse potential, biogenic amine releaser that suppresses cocaine self-administration. J Pharmacol Exp Ther 313(3):1361–1369

Rudnick G (1998) Bioenergetics of neurotransmitter transport. J Bioenerg Biomembr 30 (2):173–185

Saha K, Partilla JS, Lehner KR, Seddik A, Stockner T, Holy M et al (2015) "Second-generation" mephedrone analogs, 4-MEC and 4-MePPP, differentially affect monoamine transporter function. Neuropsychopharmacology 40(6):1321–1331

Sakloth F, Kolanos R, Mosier PD, Bonano JS, Banks ML, Partilla JS et al (2015) Steric parameters, molecular modeling and hydropathic interaction analysis of the pharmacology of para-substituted methcathinone analogues. Br J Pharmacol 172(9):2210–2218

Sanchez C, Reines EH, Montgomery SA (2014) A comparative review of escitalopram, paroxetine, and sertraline: are they all alike? Int Clin Psychopharmacol 29(4):185–196

Sandtner W, Stockner T, Hasenhuetl PS, Partilla JS, Seddik A, Zhang YW et al (2016) Binding mode selection determines the action of ecstasy homologs at monoamine transporters. Mol Pharmacol 89(1):165–175

Schicker K, Uzelac Z, Gesmonde J, Bulling S, Stockner T, Freissmuth M (2012) Unifying concept of serotonin transporter-associated currents. J Biol Chem 287(2):438–445

Schindler CW, Thorndike EB, Goldberg SR, Lehner KR, Cozzi NV, Brandt SD et al (2016) Reinforcing and neurochemical effects of the "bath salts" constituents 3,4-methylenedioxypyrovalerone (MDPV) and 3,4-methylenedioxy-N-methylcathinone (methylone) in male rats. Pyschopharmacology (Berl) 233(10):1981–1990

Scholze P, Zwach J, Kattinger A, Pifl C, Singer EA, Sitte HH (2000) Transporter-mediated release: a superfusion study on human embryonic kidney cells stably expressing the human serotonin transporter. J Pharmacol Exp Ther 293(3):870–878

Schütte J (1961) Anorexigenic propiophenones. Temmler Werke, Hamburg-Neugraben, Germany. US3001910A

Seddik A, Geerke DP, Stockner T, Holy M, Kudlacek O, Cozzi NV et al (2017) Combined simulation and mutation studies to elucidate selectivity of unsubstituted amphetamine-like cathinones at the dopamine transporter. Mol Inform 36(5–6)

Seeger E (1967) α-Pyrrolidino ketones. Boehringer Ingelheim GmbH, Biberach an der Riss, Germany. Boehringer Ingelheim G.m.b.H. US3314970

Shanks KG, Dann T, Behonick G, Terrell A (2012) Analysis of first and second-generation legal highs for synthetic cannabinoids and synthetic stimulants by ultra-performance liquid chromatography and time of flight mass spectrometry. J Anal Toxicol 36(6):360–371

Shekar A, Aguilar JI, Galli G, Cozzi NV, Brandt SD, Ruoho AE et al (2017) Atypical dopamine efflux caused by 3,4-methylenedioxypyrovalerone (MDPV) via the human dopamine transporter. J Chem Neuroanat 83-84:69–74

Shimada S, Kitayam S, Lin CL, Patel A, Nanthakumar E, Gregor P et al (1991) Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. Science 254 (5031):576–578

Shortall SE, Macerola AE, Swaby RT, Jayson R, Korsah C, Pillidge KE et al (2013) Behavioural and neurochemical comparison of chronic intermittent cathinone, mephedrone and MDMA administration to the rat. Eur Neuropsychopharmacol 23(9):1085–1095

Shortall SE, Spicer CH, Ebling FJ, Green AR, Fone KC, King MV (2016) Contribution of serotonin and dopamine to changes in core body temperature and locomotor activity in rats following repeated administration of mephedrone. Addict Biol 21(6):1127–1139

Sikk K, Taba P (2015) Methcathinone "kitchen chemistry" and permanent neurological damage. Int Rev Neurobiol 120:257–271

Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu LH, Huwyler J et al (2013) Pharmacological characterization of designer cathinones in vitro. Br J Pharmacol 168(2):458–470

Sitte HH, Huck S, Reither H, Boehm S, Singer EA, Pifl C (1998) Carrier-mediated release, transport rates, and charge transfer induced by amphetamine, tyramine, and dopamine in mammalian cells transfected with the human dopamine transporter. J Neurochem 71(3):1289–1297

Sitte HH, Scholze P, Schloss P, Pifl C, Singer EA (2000) Characterization of carrier-mediated efflux in human embryonic kidney 293 cells stably expressing the rat serotonin transporter: a superfusion study. J Neurochem 73(3):1317–1324

Sitte HH, Freissmuth M (2015) Amphetamines, new psychoactive drugs and the monoamine transporter cycle. Trends Pharmacol Sci 36(1):41–50

Solis E Jr (2017) Electrophysiological actions of synthetic cathinones on monoamine transporters. Curr Top Behav Neurosci 32:73–92

Sonders MS, Amara SG (1996) Channels in transporters. Curr Opin Neurobiol 6(3):294–302

Sonders MS, Zhu SJ, Zahniser NR, Kavanaugh MP, Amara SG (1997) Multiple ionic conductances of the human dopamine transporter: actions of dopamine and psychostimulants. J Neurosci 17 (3):960–974

Spiller HA, Ryan ML, Weston RG, Jansen J (2011) Clinical experience with and analytical confirmation of "bath salts" and "legal highs" (synthetic cathinones) in the United States. Clin Toxical (Phila) 49(6):499–505

Stepens A, Logina I, Liguts V, Aldins P, Eksteina I, Platkajis A et al (2008) A Parkinsonian syndrome in methcathinone users and the role of manganese. N Engl J Med 358(10):1009–1017

Suplicy H, Boguszewski CL, dos Santos CM, do Desterro de Figueiredo M, Cuha DR, Radominski R (2014) A comparative study of five centrally acting drugs on the pharmacological treatment of obesity. Int J Obes (Lond) 38(8):1097–1103

Suyama JA, Sakloth F, Kolanos R, Glennon RA, Lazenka MF, Negus SS et al (2016) Abuse-related neurochemical effects of para-substituted methcathinone analogs in rats: microdialysis studies of nucleus accumbens dopamine and serotonin. J Pharmacol Exp Ther 356(1):182–190

Suzuki M, Deschamps JR, Jacobson AE, Rice KC (2015) Chiral resolution and absolute configuration of the enantiomers of the psychoactive "designer drug" 3,4-methylenedioxypyrovalerone. Chirality 27(4):287–293

Thomae K (1963) α-Pyrrolidino-ketones. Dr. Karl Thomae GmbH, Biberach an der Riss, Germany. GB 933507

United Nations Office on Drugs and Crime (UNODC) (2017) Market analysis of synthetic drugs. Amphetamine-type stimulants, new psychoactive substances. World Drug Report 2017. Booklet

4. Vienna, Austria. https://www.unodc.org/wdr2017/field/Booklet_4_ATSNPS.pdf. Accessed 8 Sep 2018
van der Schoot J, Ariens EJ, van Rossum J, Hurkmans JA (1962) Phenylisopropylamine derivatives, structure and action. Arzneimittelforschung 12:902–907
Vandewater SA, Creehan KM, Taffe MA (2015) Intravenous self-administration of entactogen-class stimulants in male rats. Neuropharmacology 99:538–545
Vaugeois JM, Bonnet JJ, Duterte-Boucher D, Costentin J (1993) In vivo occupancy of the striatal dopamine uptake complex by various inhibitors does not predict their effects on locomotion. Eur J Pharmacol 230(2):195–201
Volkow ND, Morales M (2015) The brain on drugs: from reward to addiction. Cell 162(4):712–725
Warrick BJ, Hill M, Hekman K, Christensen R, Goetz R, Casavant MJ et al (2013) A 9-state analysis of designer stimulant, "bath salt," hospital visits reported to poison control centers. Ann Emerg Med 62(3):244–251
Wander A (1963) α-Pyrrolidino-valerophenones. Dr. A. Wander S.A., Bern, Switzerland. GB 927475
Watterson LR, Hood L, Sewalia K, Tomek SE, Yahn S, Johnson CT et al (2012) The reinforcing and rewarding effects of methylone, a synthetic cathinone commonly found in "bath salts." J Addict Res Ther (Suppl 9). pii: 002
Watterson LR, Kugahl PR, Nemirovsky NE, Sewalia K, Grabenauer M, Thomas BF et al (2014) Potent rewarding and reinforcing effects of the synthetic cathinone 3,4-methylenedioxypyrovalerone (MDPV). Addict Biol 19(2):165–174
Watterson LR, Olive MF (2017) Reinforcing effects of cathinone NPS in the intravenous drug self-administration paradigm. Curr Top Behav Neurosci 32:133–143
Wee S, Anderson KG, Baumann MH, Rothman RB, Blough BE, Woolverton WL (2005) Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. J Pharmacol Exp Ther 313(2):848–854
Wee S, Woolverton WL (2006) Self-administration of mixtures of fenfluramine and amphetamine by rhesus monkeys. Pharmacol Biochem Behav 84(2):337–343
Willuhn I, Wanat MJ, Clark JJ, Phillips PE (2010) Dopamine signaling in the nucleus accumbens of animals self-administering drugs of abuse. Curr Top Behav Neurosci 3:29–71
Wojcieszak J, Andrzejczak D, Wojtas A, Golembiowska K, Zawilska JB (2018) Effects of the new generation of α-pyrrolidinophenones on spontaneous locomotor activities in mice, and on extracellular dopamine and serotonin levels in the mouse striatum. Forensic Toxicol 36 (2):334–350
Wright MJ Jr, Angrish D, Aarde SM, Barlow DJ, Buczynski MW, Creehan KM et al (2012) Effect of ambient temperature on the thermoregulatory and locomotor stimulant effects of 4-methylmethcathinone in Wistar and Sprague-Dawley rats. PLoS One 7(8):e44652
Yan Y, Newman AH, Xu M (2014) Dopamine D1 an D3 receptors mediate reconsolidation of cocaine memories in mouse models of drug self-administration. Neuroscience 278:154–164
Zdrazil B, Hellsberg E, Viereck M, Ecker GF (2016) From linked open data to molecular interaction: studying selectivity trends for ligands of the human serotonin and dopamine transporter. Medchemcomm 7(9):1819–1831
Zolkowska D, Jain R, Rothman RB, Partilla JS, Roth BL, Setola V et al (2009) Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. J Pharmacol Exp Ther 329(2):738–746

Pharmacology of MDMA- and Amphetamine-Like New Psychoactive Substances

Linda D. Simmler and Matthias E. Liechti

Contents

Abstract

New psychoactive substances (NPS) with amphetamine-, aminoindan-, and benzofuran basic chemical structures have recently emerged for recreational drug use. Detailed information about their psychotropic effects and health risks is often limited. At the same time, it emerged that the pharmacological profiles of these NPS resemble those of amphetamine or

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3,4-methylenedioxymethamphetamine (MDMA). Amphetamine-like NPS induce psychostimulation and euphoria mediated predominantly by norepinephrine (NE) and dopamine (DA) transporter (NET and DAT) inhibition and transporter-mediated release of NE and DA, thus showing a more catecholamine-selective profile. MDMA-like NPS frequently induce well-being, empathy, and prosocial effects and have only moderate psychostimulant properties. These MDMA-like substances primarily act by inhibiting the serotonin (5-HT) transporter (SERT) and NET, also inducing 5-HT and NE release. Monoamine receptor interactions vary considerably among amphetamine- and MDMA-like NPS. Clinically, amphetamine- and MDMA-like NPS can induce sympathomimetic toxicity. The aim of this chapter is to review the state of knowledge regarding these substances with a focus on the description of the in vitro pharmacology of selected amphetamine- and MDMA-like NPS. In addition, it is aimed to provide links between pharmacological profiles and in vivo effects and toxicity, which leads to the conclusion that abuse liability for amphetamine-like NPS may be higher than for MDMA-like NPS, but that the risk for developing the life-threatening serotonin syndrome may be increased for MDMA-like NPS.

Keywords

4-FA · 4-Fluoroamphetamine · 5-IT · Aminoindans · Amphetamine · Benzofurans · DAT · Dopamine · MDMA · Monoamines · NET · Noradrenaline · NPS · Release · Serotonin · SERT · Uptake

Acronyms of the Discussed New Psychoactive Substances (NPS)

2-AI	2-Aminoindane
3-MMC	3-Methyl-*N*-methylcathinone
4-APB	4-(2-Aminopropyl)benzofuran
4-FA	4-Fluoroamphetamine
4-MA	4-Methylamphetamine
4-MTA	4-Methylthioamphetamine
5-APB	5-(2-Aminopropyl)benzofuran
5-APDB	5-(2-Aminopropyl)-2,3-dihydrobenzofuran
5-EAPB	5-(2-Ethylaminopropyl)benzofuran
5-IAI	5-Iodoaminoindan
5-IT, 5-API	5-(2-Aminopropyl)indole
5-MAPDB	1-(2,3-Dihydrobenzofuran-5-yl)-*N*-methylpropan-2-amine
6-APB	6-(2-Aminopropyl)benzofuran
6-APDB	6-(2-Aminopropyl)-2,3-dihydrobenzofuran
7-APB	7-(2-Aminopropyl)benzofuran
MBDB	3,4-Methylenedioxyphenyl-*N*-methyl-2-butanamine
MDA	3,4-Methylenedioxyamphetamine
MDAI	3,4-Methylenedioxyaminoindan

MDEA	3,4-Methylenedioxy-*N*-ethylamphetamine
MMAI	5-Methoxy-6-methyl-2-aminoindan
PMA	*para*-Methoxyamphetamine
PMMA	*para*-Methoxymethamphetamine

1 Introduction

Amphetamine and its derivative 3,4-methylenedioxymethamphetamine (MDMA) are substances that have been abused for decades. Amphetamine and alternatively methamphetamine are typically sold under the street name "speed," and MDMA is the substance typically associated with "ecstasy" pills. Although MDMA is a 3,4-methylenedioxy derivative of amphetamine, the subjective effects as well as the pharmacological profiles of MDMA and amphetamine are distinct. Psychostimulation and euphoria are commonly described acute subjective effects of amphetamine consumption (Dolder et al. 2017). MDMA is the prototypical entactogenic/empathogenic drug (Nichols 1986) and induces fewer psychostimulant effects than amphetamine (Bershad et al. 2016). Enhancement of feelings of love, happiness, and closeness to others are typical entactogenic effects (Hysek et al. 2014a, b; Liechti et al. 2001). Amphetamine and MDMA act on monoamine reuptake transporters (Simmler et al. 2013; Verrico et al. 2007). By blocking the serotonin (5-HT), dopamine (DA), and norepinephrine (NE) transporters (SERT, DAT, and NET, respectively), reuptake of the respective neurotransmitters is prevented, causing increased neurotransmitter concentrations in the synaptic cleft (Kehr et al. 2011; Torres et al. 2003). Amphetamine derivatives typically also induce transporter-mediated release of neurotransmitters (Blakely et al. 2005; Hysek et al. 2012c). Since the neurotransmitters 5-HT, DA, and NE are differentially involved in modulating behavior and subjective effects, distinct pharmacological profiles of drugs of abuse can be linked to specific psychotropic effects and intoxication (Dolder et al. 2017; Liechti 2015; Schmid et al. 2014). As such, amphetamine with preference for human DAT and NET is experienced differently than MDMA, which, in contrast, preferentially acts at human SERT and NET vs. DAT (Simmler et al. 2013).

The chemical diversity found among substances commonly referred to as new psychoactive substances (NPS) is quite pronounced. Over 600 different NPS have emerged on the illicit drug market since the beginning of this century (EMCDDA 2016). The pharmacology and psychotropic effects range widely among amphetamine-based NPS. For example, subjective effects induced by NPS based on the amphetamine template may range from hallucinogenic via entactogenic to stimulant properties predominantly depending on the nature and location of substituents on the phenyl ring (Hill and Thomas 2011; Liechti 2015; Zwartsen et al. 2017; Nichols 2017). In addition, many amphetamine-based NPS carry a keto group in the ß-position of the carbon side chain, thus giving rise to the cathinone template (Fig. 1) (Prosser and Nelson 2012). Cathinone NPS are discussed in detail

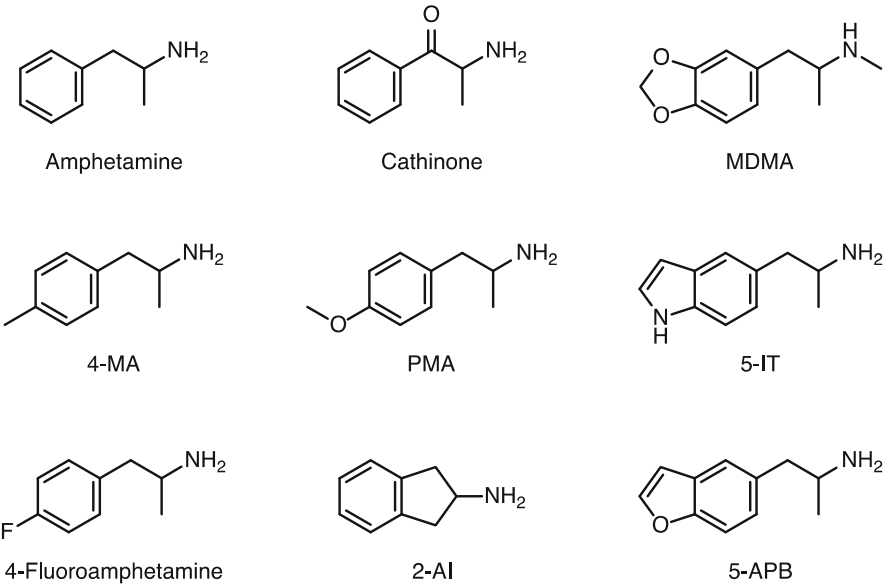

Fig. 1 Chemical structures of amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and selected new psychoactive substances (NPS)

in the preceding chapter of this book. The present chapter will focus on non-ß-keto NPS that resemble amphetamine and MDMA in their pharmacology and subjective effects. The main focus of this chapter is the description of the in vitro pharmacology of selected amphetamine- and MDMA-like NPS, with the additional aim to provide links between pharmacological profiles and in vivo effects and toxicity.

By using the terms amphetamine- and MDMA-like NPS, we refer mostly to pharmacological profiles that are comparable to amphetamine and/or MDMA. Pivotal are the relative potencies for inhibition of the human SERT, DAT, and NET. Furthermore, characteristic for amphetamine- and MDMA-like substances is that they induce transporter-mediated release of monoamines. Release is typical for amphetamine and MDMA and distinguishes amphetamine derivatives from pure uptake blockers such as cocaine or the NPS 3,4-methylenedioxypyrovalerone (MDPV; Baumann et al. 2013). Our classification of NPS as amphetamine- or MDMA-like is mainly based on the relative activity for uptake inhibition at DAT vs. SERT, referred to as DAT/SERT ratio. DAT/SERT ratios are calculated as IC_{50} value for SERT divided by IC_{50} value for DAT (also as 1/(DAT IC_{50}) divided by 1/(SERT IC_{50})). DAT/SERT ratios can also be calculated for EC_{50} values for transporter-mediated release (Baumann et al. 2012; Marusich et al. 2016). MDMA acts preferentially on SERT, reflected in a low DAT/SERT ratio. In contrast, amphetamine acts preferentially on DAT, reflected in a high DAT/SERT ratio (Simmler et al. 2013). Below, we will discuss the pharmacology and psychotropic effects of amphetamine- and MDMA-like NPS separately.

2 3,4-Methylenedioxymethamphetamine- and Amphetamine-Like New Psychoactive Substances

The amphetamine derivatives discussed in this chapter are *N*-ethylamphetamine, 4-fluoroamphetamine (4-FA), 4-fluoromethamphetamine, 5-(2-aminopropyl)indole (5-IT, also known as 5-API), 4-methylamphetamine (4-MA), 3,4-methylenedioxyphenyl-*N*-methyl-2-butanamine (MBDB), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), 4-methylthioamphetamine (4-MTA), *para*-methoxyamphetamine (PMA), and *para*-methoxymethamphetamine (PMMA). Aminoindans discussed are 2-aminoindane (2-AI), 5-iodoaminoindan (5-IAI), 3,4-methylenedioxyaminoindan (MDAI), and 5-methoxy-6-methyl-2-aminoindan (MMAI). Benzofurans discussed are 4-(2-aminopropyl)benzofuran (4-APB), 5-(2-aminopropyl)benzofuran (5-APB), 5-(2-aminopropyl)-2,3-dihydrobenzofuran (5-APDB), 5-(2-ethylaminopropyl)benzofuran (5-EAPB), 1-(2,3-dihydrobenzofuran-5-yl)-*N*-methylpropan-2-amine (5-MAPDB), 6-(2-aminopropyl)benzofuran (6-APB), 6-(2-aminopropyl)-2,3-dihydrobenzofuran (6-APDB), and 7-(2-aminopropyl)benzofuran (7-APB).

If not otherwise noted, we refer to the racemic mixtures of compounds, except for D- or (*S*)-(+)-amphetamine. The isomers of psychoactive drugs with asymmetric centers can have different biological activity (Baumann et al. 2007). However, since street drugs are produced as racemic mixtures, pharmacological in vitro- or animal studies that use the racemic mixtures of the compounds are representative reflections of street drug activity. Chemical structures of amphetamine, MDMA, and selected NPS are shown in Fig. 1. Cathinone derivatives are discussed in the preceding chapter of this book, but the structure of cathinone is displayed in Fig. 1 to illustrate the ß-keto substituent typical for cathinone-based NPS. 2-AI forms the basic structure of aminoindan-derived NPS, and 5-APB is shown as example for benzofuran NPS.

3 3,4-Methylenedioxymethamphetamine-Like New Psychoactive Substances

3.1 Pharmacology of 3,4-Methylenedioxymethamphetamine-Like Substances

Several specific ring-substituted amphetamine-based NPS that lack the ß-keto substituent resemble MDMA in their pharmacological profile. MDMA-like NPS potently inhibit the NET and SERT with lower potency for DAT inhibition. Accordingly, their DAT/SERT ratio is low, comparable to MDMA, for which our laboratory reported a DAT/SERT ratio of 0.08 (Simmler et al. 2013). DAT/SERT ratios for MDMA below 1 were also reported for release (Eshleman et al. 2017;

Marusich et al. 2016; Baumann et al. 2012). As for uptake inhibition (Simmler et al. 2013), these studies also report DAT/SERT ratios for amphetamine- or methamphetamine-induced release that are >150 times higher than the DAT/SERT ratio of MDMA. A low DAT/SERT ratio predicts MDMA-like subjective effects and a lower dependence potential compared to amphetamine (Suyama et al. 2016; Liechti 2015). MDMA-like pharmacological properties can be found among benzofurans, aminoindans, and amphetamines.

MDMA is a prototypical entactogenic/empathogenic drug that retains some psychostimulant effects. It increases empathy, sociability, closeness to others, but also happiness and self-esteem (Hysek et al. 2014a, b; Liechti et al. 2001). Cardiostimulant effects are common and include increased blood pressure, increased heart rate, and hyperthermia (Vizeli and Liechti 2017). Bruxism resulting from increased muscle tension is also experienced frequently (Cole and Sumnall 2003). The psychotropic and cardiostimulant effects have been attributed foremost to the 5-HT and NE release properties of MDMA (Hysek et al. 2011, 2012c). Concomitant to inducing transporter-mediated 5-HT and NE release, MDMA inhibits the reuptake of the respective neurotransmitters at SERT and NET. MDMA inhibits DAT with significant lower potency than it inhibits NET and SERT. Its low DAT/SERT ratio (0.08; Simmler et al. 2013) is a representative measure for selectivity of SERT over DAT and is used here to compare NPS to MDMA.

MDMA is also a low-potency partial agonist of the 5-HT_{2A} receptor. Although not frequent, mild hallucinogen-like effects of MDMA have been reported, which may be attributable to 5-HT_{2A} agonism (Nichols 2004; Liechti et al. 2000). MDA, the active metabolite of MDMA (Hysek et al. 2011), shows a tenfold higher potency for 5-HT_{2A} agonism than MDMA (Rickli et al. 2015c). MDA likely contributes to the mode of action of MDMA and might contribute to the mild hallucinogenic effects of MDMA.

Binding affinity of MDMA for adrenergic receptors is low, but since MDMA increases NE levels via transporter-mediated NE release and NET uptake inhibition, indirectly NE-mediated effects at adrenergic receptors clearly contribute to MDMA action (Hysek et al. 2011). β-Adrenoceptors are involved in MDMA-induced increase of heart rate (Hysek et al. 2010). The α_1- and β-adrenoceptors have been implicated in hyperthermia and drug-induced vasoconstriction (Hysek et al. 2012a). α_{2A}-Adrenocepors are associated with sympathomimetic toxicity and augmented NE release (Hysek et al. 2012b). Potent transporter-mediated NE release or even NET inhibition seems sufficient to induce cardiostimulant effects mediated through the different adrenergic receptors (Hysek et al. 2011). NPS with potent effects at NET thus likely induce psychostimulation and sympathomimetic toxicity.

3.1.1 Serotonergic Toxicity

Increased levels of 5-HT can lead to serotonergic toxicity and, in extreme cases, can result in precipitation of the serotonin syndrome. Typical symptoms of the serotonin syndrome include neuromuscular hyperactivity, clonus, autonomous

hyperactivity (including hyperthermia), sweating, agitation, and confusion (Gillman 2005; Liechti 2015). MDMA-like drugs typically induce symptoms of serotonergic intoxication, potentially leading to a severe serotonin syndrome including hyperthermia but also a syndrome of inadequate diuretic hormone (SIADH) resulting in hyponatremia (Simmler et al. 2011; Hartung et al. 2002; Holden and Jackson 1996). Hyperthermia, followed by life-threatening complications such as rhabdomyolysis, intravascular coagulation, and organ failure, is commonly involved in fatal intoxications with psychostimulants (Cole and Sumnall 2003). Although hyperthermia is a well-described unwanted effect of MDMA (Liechti 2014), the past has shown that certain psychoactive substances induce hyperthermia more readily than MDMA, which has been associated with fatal complications. For example, the more traditional substances PMA, PMMA, and 4-MTA show a selectivity for SERT over DAT similar to MDMA (Table 1) but the morbidity and mortality linked to these particular substances has been observed to be greater and more distinct compared to MDMA (Lurie et al. 2012; Vevelstad et al. 2012). Inhibition of monoamine oxidase (MAO)-A by PMA, PMMA, and 4-MTA (Matsumoto et al. 2014) might play a major role in the induction of severe hyperthermia. Increased levels of cytosolic 5-HT, resulting from MAO inhibition, might augment drug-induced release of 5-HT via SERT. The combination of MAO inhibition and 5-HT release, as reported for PMA, PMMA, and 4-MTA (Matsumoto et al. 2014), is therefore particularly prone to induce potentially life-threatening serotonergic intoxication. MAO inhibition has been implicated in hyperthermia and the life-threatening serotonin syndrome (Carmo et al. 2003; Gillman 2005). From the use of selective SERT inhibitors as antidepressants, it is well known that the combination of SERT inhibition together with MAO inhibition can cause life-threatening serotoninergic intoxication, including hyperthermia. Testing NPS for the potential of MAO inhibition might therefore warrant further investigation.

3.1.2 Noradrenergic Toxicity

Sympathomimetic toxicity results from increased activation of the NE system, either via direct activation of adrenergic receptors or indirectly via receptor activation due to increased NE levels (Hysek et al. 2010, 2011, 2012a). Hyperthermia, hypertension, tachycardia, and agitation are typical symptoms of stimulant-induced noradrenergic toxicity (Cruickshank and Dyer 2009; Cole and Sumnall 2003). Cardiovascular sympathomimetic toxicity is typically associated with amphetamine but also occurs following MDMA administration via induction of NE release and increases in plasma NE levels. NE-mediated hyperthermia involves stimulation of α_1- and $ß_3$-adrenoceptors (Sprague et al. 2004; Hysek et al. 2012a). Mechanistically, hyperthermia occurs by activation of α_1-adrenoceptors via increased vasoconstriction, leading to decreased heat dissipation. $ß_3$-Adrenoceptor activation causes mitochondrial uncoupling, which increases heat generation (Liechti 2014).

Table 1 Uptake inhibition potencies of MDMA, amphetamine, methamphetamine, and selected MDMA- and amphetamine-like NPS in alphabetical order

	NET	DAT	SERT		
	IC_{50} (μM) (95% CI)	IC_{50} (μM) (95% CI)	IC_{50} (μM) (95% CI)	DAT/SERT ratios	Values from
2-AI	0.54 (0.42–0.69)	58 (4–905)	>100	>1	Simmler et al. (2014b)
D-amphetamine	0.094 (0.06–0.14)	1.30 (0.83–2.0)	>10	>10	Simmler et al. (2013)
4-APB	0.24 (0.2–0.3)	12 (9–16)	5.5 (3.4–8.7)	0.46	Rickli et al. (2015b)
5-APB	0.16 (0.08–0.3)	6.1 (4–9)	0.29 (0.17–0.5)	0.05	Rickli et al. (2015b)
6-APB	0.19 (0.1–0.3)	3.3 (2.4–4.5)	0.93 (0.7–1.3)	0.29	Rickli et al. (2015b)
7-APB	0.27 (0.2–0.3)	20 (16–26)	13 (9–18)	0.65	Rickli et al. (2015b)
5-APDB	0.29 (0.2–0.5)	49 (33–73)	0.58 (0.4–0.9)	0.01	Rickli et al. (2015b)
6-APDB	0.56 (0.4–0.8)	33 (25–43)	2.3 (1.4–3.9)	0.07	Rickli et al. (2015b)
5-EAPB	0.56 (0.4–0.7)	4.9 (3–8)	0.72 (0.5–1.1)	0.15	Rickli et al. (2015b)
N-ethylamphetamine	0.20 (0.15–0.27)	5.86 (4.8–7.1)	8.77 (6–13)	1.5	Simmler et al. (2014a)
4-Fluoroamphetamine	0.20 (0.14–0.28)	3.7 (2.4–5.7)	19 (11–33)	5.1	Rickli et al. (2015a)
4-Fluoromethamphetamine	0.22 (0.14–0.35)	7.7 (2.5–24)	8.7 (3.8–20)	1.1	Rickli et al. (2015a)
5-IAI	0.76 (0.60–0.98)	23 (15–35)	2.5 (1.9–3.4)	0.11	Simmler et al. (2014b)
5-IT	0.04 (0.03–0.06)	0.68 (0.55–0.85)	1.3 (0.9–1.7)	1.9	Luethi et al. (2017)
5-MAPDB	0.96 (0.5–1.7)	77 (62–96)	1.2 (0.7–2)	0.02	Rickli et al. (2015b)
MBDB	2.80 (1.9–4.1)	22 (20–26)	2.04 (1.4–3.0)	0.09	Simmler et al. (2013)
MDA	0.42 (0.3–0.6)	20.5 (20.3–20.6)	4.9 (3.5–6.8)	0.24	Rickli et al. (2015b)
MDAI	0.65 (0.50–0.84)	31 (23–41)	8.3 (3.2–22)	0.2	Simmler et al. (2014b)
MDEA	1.02 (0.78–1.3)	9.3 (8.0–11)	1.27 (0.93–1.7)	0.14	Simmler et al. (2013)
MDMA	0.447 (0.33–0.60)	17 (12–24)	1.36 (1.0–2.0)	0.08	Simmler et al. (2013)
D-methamphetamine	0.064 (0.04–0.09)	1.05 (0.74–1.5)	>10	>10	Simmler et al. (2013)
4-Methylamphetamine	0.31 (0.24–0.42)	5.6 (4.5–6.9)	0.82 (0.64–1.05)	0.15	Luethi et al. (2017)
MMAI	3.6 (2.5–5.3)	193 (167–225)	0.68 (0.50–0.92)	0.004	Luethi et al. (2017)
4-MTA	1.52 (1.3–1.9)	22 (15–32)	0.54 (0.37–0.80)	0.02	Simmler et al. (2014a)
PMA	0.80 (0.50–1.0)	71 (60–83)	2.37 (2.0–2.9)	0.03	Simmler et al. (2014a)
PMMA	1.20 (0.75–1.8)	49 (18–135)	1.77 (1.1–2.9)	0.04	Simmler et al. (2014a)

Experiments were conducted in vitro with cultured cells that express the human NET, DAT, or SERT. All substances were tested as racemic mixtures except for D-amphetamine

3.2 3,4-Methylenedioxymethamphetamine-Like Amphetamine Derivatives

The amphetamine derivatives MBDB, 4-MA, MDEA, 4-MTA, PMA, and PMMA show uptake inhibition profiles and DAT/SERT ratios similar to MDMA (Table 1). In vivo drug-discrimination experiments in rats suggest that PMA, PMMA, and 4-MTA show MDMA-like properties (Dukat et al. 2002; Glennon et al. 2007). However, these substances did not substitute for amphetamine in the drug-discrimination experiments, which predicts that PMA, PMMA, and 4-MTA have less stimulant-like properties than MDMA. The (*S*)-(+)-isomer of MDMA showed stimulant properties in rats, reflected in hyperlocomotion and substitution for amphetamine in drug-discrimination tests (Glennon et al. 1988). 4-MTA, PMA, and PMMA have been associated with severe serotonergic toxicity (Liechti 2015). The combination of MAO inhibition and potent 5-HT release properties are likely the cause for the high morbidity and mortality reported for these substances. Similarly, 4-MA is a potent 5-HT releaser (Baumann et al. 2011) and inhibits MAO, and has been associated with fatal intoxications (Blanckaert et al. 2013). Furthermore, 4-MA is a potent partial agonist at the 5-HT_{2B} receptor (Luethi et al. 2017). This receptor has been implicated in endocardial fibrosis (Roth 2007) but whether chronic substance use indeed causes such cardiac complications remains to be established.

MDEA, although equal to MDMA for SERT inhibition potency (Table 1), induces hyperthermia in rats less potently than MDMA (Colado et al. 1999). The lower potency at NET compared to MDMA (Table 1) might account for this difference, since NE release plays a crucial role in the induction of drug-induced hyperthermia (Sprague et al. 2004; Hysek et al. 2012a; Liechti 2014). Similarly, MBDB is less potent at NET inhibition than MDMA (Table 1; or in rat synaptosomes $IC_{50(MDMA)} = 405$ nM, $IC_{50(MBDB)} = 1{,}233$ (Johnson et al. 1991)). MBDB is considered to share a range of psychopharmacological effects also observed with MDMA and drug-discrimination studies revealed that MBDB substitutes for MDMA (Aerts et al. 2000). To the best of our knowledge, no studies on the effect of MBDB and MDEA on MAO exist to date.

3.3 3,4-Methylenedioxymethamphetamine-Like Aminoindans

Aminoindans were originally developed as potential therapeutic bronchodilators but have emerged in recent years as NPS among recreational drug users, although with relatively low prevalence (Brandt et al. 2013; Sainsbury et al. 2011). Among the relatively few NPS falling into the aminoindan class, differences in the pharmacological profiles have been described. The aminoindans 5-IAI, MDAI, and MMAI show an MDMA-like uptake inhibition profile with DAT/SERT ratios smaller or equal to 0.2 (Table 1). In contrast, 2-aminoindan (2-AI) and *N*-methyl-2-AI are selective NET inhibitors and NE releasers, with 2-AI also releasing DA (Simmler et al. 2014b; Luethi et al. 2017). MDAI induces transporter-mediated release of

5-HT and NE, similar to MDMA, but, unlike MDMA, MDAI does not induce DA release through the human DAT under the conditions investigated (Simmler et al. 2014b; Eshleman et al. 2017). 5-IAI causes 5-HT and DA release, but not NE release, although it acts as a potent NET inhibitor (Iversen et al. 2013; Simmler et al. 2014b). According to user reports, MDAI and 5-IAI cause euphoria and have entactogenic properties (Pinterova et al. 2017). Interestingly, MMAI was shown to be selective for SERT over DAT and NET when inducing uptake inhibition and transporter-mediated release (Johnson et al. 1991; Luethi et al. 2017). The lack of NET activity predicts that MMAI might not be truly experienced as MDMA-like, since NE-mediated psychostimulation is likely absent or weak in acute MMAI effects, whereas MDMA induces pronounced NE-mediated psychostimulation (Hysek et al. 2010, 2011, 2012a). The selective serotonergic activity could imply a high risk for serotonin syndrome, similar to 4-MTA. However, unlike 4-MTA, MMAI does not significantly inhibit MAO (Scorza et al. 1999), which might be relevant when considering the potential for severe adverse effects.

Some aminoindans were developed as potential non-neurotoxic alternatives for MDMA. MDAI was reported to substitute for MDMA, but not LSD, in drug-discrimination studies in rats. However, in contrast to MDMA, MDAI did not cause 5-HT depletion (Nichols et al. 1990). Similarly, no indication for 5-HT toxicity was found for 5-IAI (Nichols et al. 1991). Non-neurotoxic effects of MDAI and 5-IAI suggested that these aminoindans might display a safer risk profile compared to MDMA but recent animal studies indicated that MDAI can induce potentially life-threatening toxicity related to the serotonin syndrome (Gatch et al. 2016; Palenicek et al. 2016). Three fatal intoxications involving MDAI and other substances have been described and from the antemortem information available in one case, the involvement of serotonin toxicity was considered likely (Corkery et al. 2013).

The receptor interaction profiles of MDMA-like aminoindans show that 5-IAI exhibits nanomolar affinity at the 5-HT_{1A}, 5-HT_{2A} (Simmler et al. 2014b), and the 5-HT_{2B} receptors (Iversen et al. 2013). Furthermore, affinity ($K_i = 1.2$ μM) for 5-IAI was also observed at the 5-HT_{2C} receptor (Simmler et al. 2014b). This is in contrast to MDAI, which did not exhibit affinity to these 5-HT receptors, and to MMAI, which only had activity in the micromolar range (Luethi et al. 2017).

3.4 3,4-Methylenedioxymethamphetamine-Like Benzofurans

The pharmacology and toxicology of benzofurans is relatively poorly explored to date, but fatal, analytically confirmed intoxication with the benzofuran 5-APB alone or in combination with 3-MMC has been reported (Adamowicz et al. 2014; McIntyre et al. 2015). Benzofurans were described by users as substances inducing entactogenic and stimulant effects, but also sympathomimetic toxicity, including hyperthermia (Welter-Luedeke and Maurer 2016). In drug-discrimination studies in rats, the 2,3-dihydrobenzofurans 5-APDB and 6-APDB substituted for MDMA-like entactogens, but not for amphetamine (Monte et al. 1993).

Many benzofurans show MDMA-like pharmacological profiles. Rickli et al. (2015b) have characterized a set of benzofurans, which were all potent NET inhibitors and showed DAT/SERT ratios <1 (Table 1). 5-APDB, 5-MAPDB, 5-APB, and 6-APDB showed high selectivity for SERT over DAT inhibition with DAT/SERT ratios of 0.01–0.07. 5-EAPB and 6-APB have DAT/SERT ratios of 0.15 and 0.29, respectively. The least selective compounds for SERT over DAT were 4-APB and 7-APB with DAT/SERT ratios of 0.46 and 0.65. All benzofurans characterized in this study induced transporter-mediated release of one, two, or all three monoamines.

Interestingly, most benzofurans were partial agonists at the 5-HT_{2A} and the 5-HT_{2B} receptors. 5-HT_{2A} agonism of benzofurans is comparable to MDMA (activation potency of 6 μM and 55% efficacy), but MDMA does not have functional activity at the 5-HT_{2B} receptor (Rickli et al. 2015b). Since activation of the 5-HT_{2B} receptor has been associated with heart valve fibrosis (Roth 2007), chronic consumption of benzofurans might pose a risk for long-term cardiotoxicity (Dawson et al. 2014). 4-APB, 6-APB, 6-APBP, and 7-APB showed affinity for the α_{2A}-adrenoceptor in the nanomolar range (K_i values of 140–870 nM; Rickli et al. 2015b), which might contribute to the sympathomimetic toxicity by augmenting vesicular NE release (Hysek et al. 2012b).

7-APB is a moderately potent human trace amine-associated receptor 1 (TAAR1) receptor agonist with an EC_{50} in the nanomolar range (630 nM), similar to the endogenous ligands ß-PEA (260 nM) and *p*-tyramine (990 nM) and more than tenfold more potent than other benzofurans (Simmler et al. 2016). The activation of human TAAR1 might diminish the effects of psychostimulation and intoxication arising from 7-APB effects on monoamine transporters (see Sect. 4.1.3 for more details). Affinity to mouse and rat TAAR1 has been shown for many psychostimulants, but species differences are common (Simmler et al. 2016). For example, 5-IT and 4-MA bind and activate TAAR1 in the nanomolar range but do not activate human TAAR1.

3.5 Conclusions

Although MDMA-like substances have low abuse liability due to their selectivity for SERT over DAT, the risk for potentially life-threatening intoxication appears high considering serotonergic toxicity that, for some MDMA-like NPS, might be augmented due to inhibition of MAO. Sympathomimetic toxicity arising from NE action is also common. The classification into MDMA-like NPS is approximately based on DAT/SERT ratios and does not account for subtle differences in the pharmacology of NPS, such as receptor interactions or increased prevalence of 5-HT toxicity. Consequently, collecting clinical and preclinical information for each MDMA-like NPS is helpful for contextualizing the clinical features seen in acute toxicity cases.

4 Amphetamine-Like New Psychoactive Substances

4.1 Pharmacology of Amphetamine-Like Substances

Subjective effects of amphetamine involve psychostimulation, euphoria, and increased arousal. Other clinical features include acute cardiotoxicity, such as hypertension, increased blood pressure, heart rate, and body temperature (Dolder et al. 2017). Regular amphetamine consumption bears a considerable risk for abuse and dependence. Amphetamine increases DA and NE levels by DAT and NET inhibition and induction of transporter-mediated release (Heal et al. 2013). Amphetamine further shows moderate affinity for the α_{2A}-adrenoceptor and the 5-HT_{1A} receptor (Simmler et al. 2013) and is a TAAR1 ligand (Bunzow et al. 2001) with potent full-agonist properties at the human TAAR1 (Simmler et al. 2016).

Non-ß-keto NPS described here as amphetamine-like show relatively good uptake inhibition potencies at SERT with DAT/SERT ratios between 1 and 6 (Table 1). In comparison to amphetamine or methamphetamine, 4-fluoroamphetamine, 4-fluoromethamphetamine, *N*-ethylamphetamine, and 5-IT are less selective at DAT and NET and rather nonselective for all monoamine transporters, although about tenfold more potent at NET than DAT and SERT. Like amphetamine, these amphetamine-like NPS also act as monoamine releasers (Simmler et al. 2014a; Rickli et al. 2015a; Luethi et al. 2017). In contrast, there are several cathinone derivatives with DAT/SERT ratios >10, such as cathinone, methcathinone, or 3-fluoromethcathinone (Simmler et al. 2013, 2014a), which resemble amphetamine more closely in their transporter inhibition profile.

4.1.1 Acute Dopaminergic Toxicity

Induction of rapid increase of DA level in the mesolimbic DA system is a typical acute effect of many drugs of abuse (Kehr et al. 2011), including amphetamine and cocaine, but also opioids, which have indirect effects onto the DA system (Luscher and Malenka 2011). Drug-induced increase of DA levels activates the reward system and causes euphoria (Heal et al. 2013; Sulzer 2011). Unwanted drug effects such as psychotic states and aggression can also be attributed to excessive/chronic stimulation of dopaminergic action (Harro 2015). Life-threatening excited delirium syndrome has been associated with acute dopaminergic toxicity (Mash et al. 2009).

4.1.2 Abuse Liability

The dopamine system is crucially involved in plasticity underlying drug dependence and compulsive drug use (Luscher and Malenka 2011; Pascoli et al. 2015; Koob and Volkow 2016). Substances acting to increase DA levels via DAT inhibition and/or DA reverse transport might therefore cause dependence, possibly progressing to addiction. However, the 5-HT system can oppose dopaminergic effects (Daw et al. 2002; Alex and Pehek 2007) and serotonergic properties can lower the abuse liability of psychostimulants (Simmler et al. 2017; Suyama et al. 2016; Liechti 2015; Bauer et al. 2013; Rothman and Baumann 2006; Wee et al. 2005). Accordingly, the relative action at DAT vs. SERT is crucial for assessing abuse liability of psychostimulants

(Baumann et al. 2012). From a preclinical perspective, high DAT/SERT ratios are generally considered indicative of a significant potential for abuse and dependence. The in vitro selectivity for DAT vs. SERT predicts the DA vs. 5-HT release as measured using in vivo microdialysis and also correlates with measures of reward such as intracranial self-stimulation thresholds as shown for a series of *para*-ring-substituted cathinones (Suyama et al. 2016). In addition, cathinones with a predominant action on the DA system are self-administered more readily than substances with a more 5-HT activating profile (Bonano et al. 2014; Schindler et al. 2016; Gannon et al. 2018). MDMA shows a relatively low abuse liability and has high selectivity of SERT over DAT. In the assays carried out in the authors' laboratory, the abuse dependence liability of cocaine is associated with a DAT/SERT ratio of 3.1 and amphetamine and methamphetamine have an even higher DAT/SERT ratio of >10 (Simmler et al. 2013). The amphetamine-like NPS discussed here show DAT/SERT ratios of 1–5. From the perspective of these studies, these amphetamine-like NPS might show lower abuse liability than amphetamine. However, other factors such as drug kinetics, receptor interactions, routes of administration, and social context also play important roles for the clinical picture.

4.1.3 Activation of Trace Amine-Associated Receptor 1

TAAR1 is a target of amphetamine and many amphetamine derivatives. TAAR1 is involved in the regulation of DA activity (Bradaia et al. 2009) and activation of TAAR1 reduces the abuse liability of drugs such as cocaine (Pei et al. 2014, 2015). Amphetamine and MDMA induce more pronounced psychostimulant effects in rodents not expressing TAAR1 (Lindemann et al. 2008; Di Cara et al. 2011). Psychostimulants, which act directly on TAAR1, can therefore induce negative modulation of psychotropic effects. TAAR1 activation might have protective effect with respect to drug toxicity and abuse liability. However, species differences between the rodent TAAR1 and human TAAR1 are frequent (Simmler et al. 2016). MDMA activates rat and mouse TAAR1 with low micromolar potencies, but its activation potency for the human TAAR1 is very low. Amphetamine, in contrast, activates human TAAR1 with an EC_{50} of 2.8 μM (Simmler et al. 2016). For the present chapter, available evidence on the activity of NPS at the human TAAR1 are presented.

4.2 4-Fluoroamphetamine, 4-Fluoromethamphetamine, and *N*-Ethylamphetamine

4-FA is a popular NPS and described by users to induce a mixture of amphetamine- and MDMA-like effects, which include stimulation, euphoria, and empathy (Linsen et al. 2015; Hondebrink et al. 2017). Despite the entactogenic properties reported for 4-FA, which are typical for MDMA-like substances, 4-FA has been included in this section due to its DAT/SERT ratio above a value of 1 (Wee et al. 2005; Rickli et al. 2015a; Eshleman et al. 2017). 4-FA inhibits monoamine transporters

with potencies in the rank order NET > DAT > SERT (Table 1; Eshleman et al. 2017). The same rank order has also been described for the potency of 4-FA to induce monoamine release (EC_{50} values of 28 nM (NE), 52 nM (DA), and 939 nM (5-HT); Wee et al. 2005). Cardiotoxicity and hyperthermia are typical symptoms of sympathomimetic intoxication associated with 4-FA use, and severe headache has been increasingly reported (Hondebrink et al. 2017). Cerebral hemorrhage and severe cardiovascular toxicity were diagnosed in several cases of severe or fatal intoxications (Wijers et al. 2017; Hondebrink et al. 2017). 4-FA exhibits moderate affinity for the α_{2A}-adrenoceptor and shows weak to moderate binding affinity or activation potency at the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. 4-FA is also a partial agonist at the human TAAR1 (Rickli et al. 2015a).

4-Fluoromethamphetamine is less frequently reported but resembles 4-FA in its pharmacological profile at monoamine transporters and receptors (Table 1; Rickli et al. 2015a). The in vitro data suggest that clinical effects and toxicity might be similar to those reported for 4-FA. *N*-Ethylamphetamine induces hyperlocomotion in mice (Tessel et al. 1975) and substitutes for amphetamine in drug-discrimination tests in rhesus monkeys (Woolverton and English 1997), which indicates stimulant-like properties of *N*-ethylamphetamine. Furthermore, *N*-ethylamphetamine showed reinforcing properties in rhesus monkeys (Tessel and Woods 1975). *N*-Ethylamphetamine was found to show a comparable uptake inhibition profile as 4-fluoromethamphetamine (Table 1) and functioned as a releaser of DA, NE, and 5-HT (EC_{50} values of 2.6 μM (NE), 93 μM (DA), and 43 μM (5-HT); Tessel and Rutledge 1976). *N*-Ethylamphetamine has moderate binding affinity for the α_{2A}-, 5-HT_{2A}, and 5-HT_{2C} receptors (Simmler et al. 2014a) but does not activate human TAAR1 (Simmler et al. 2016). Similar to 4-fluoromethamphetamine, the pharmacological profile for *N*-ethylamphetamine predicts effects and clinical features to be similar to 4-FA.

4.3 5-(2-Aminopropyl)Indole (5-IT)

5-IT (5-API) has caused a considerable number of fatal intoxications since its emergence in 2012 (EMCDDA 2014). The clinical cases presented with sympathomimetic and serotonergic toxicity, including hyperthermia, cardiotoxicity, and organ failure (Bäckberg et al. 2014). 5-IT inhibits NET, DAT, and SERT and 5-IT induces transporter-mediated release of NE, DA, and 5-HT (Marusich et al. 2016; Luethi et al. 2017). Although 5-IT acts more potently at NET and DAT than at SERT, serotonergic toxicity has been implicated in the intoxication cases. In addition, 5-IT inhibits MAO-A (Herraiz and Brandt 2014), which can augment the rise of 5-HT levels and poses the risk for resulting in the development of the serotonin syndrome. 5-IT has affinity for the α_{1A}- and α_{2A}-adrenoceptors (K_i of 5.4 and 1.7 μM, respectively; Luethi et al. 2017), which might contribute to sympathomimetic toxicity. 5-IT is also a potent partial agonist at 5-HT_{2A} and 5-HT_{2B} receptors (EC_{50} of 0.5 and 1.5 μM, respectively; Luethi et al. 2017), which are important for mediating

hallucinogenic effects via 5-HT_{2A} activity (Nichols 2004) and, in the long-term use, might result in heart valve fibrosis mediated via 5-HT_{2B} activity (Roth 2007).

4.4 2-Aminoindan

The use and reported fatalities of 2-AI are relatively rare (Elliott and Evans 2014), and not much more recent information about its in vivo pharmacology and toxicology could be identified. It is worth noting that the in vitro pharmacological profile of 2-AI is distinct from the MDMA-like aminoindans 5-IAI, MDAI, and MMAI (see Sect. 3.3). In contrast to the MDMA-like aminoindans, 2-AI did not inhibit SERT (Table 1; Simmler et al. 2014b). 2-AI selectively inhibits NET, also inducing NE release, and at higher concentrations, also acts as a DAT inhibitor and releaser (Simmler et al. 2014b). The selectivity of 2-AI for NET over DAT at the human transporters implies that it causes psychotropic effects that may be distinct from amphetamine, since DA-mediated euphoria might be low or absent for 2-AI. However, in rat synaptosomes, 2-AI showed DAT and NET inhibition (Horn and Snyder 1972). Amphetamine-like properties of 2-AI were also indicated behaviorally in rats by induction of hyperlocomotion (Mrongovius et al. 1978) and by substitution for amphetamine in drug-discrimination experiments (Glennon et al. 1984). Sympathomimetic effects of 2-AI can arise from increased NE levels due to NET inhibition and NE release, and high-affinity ($K_i = 450$ nM) binding of 2-AI to the α_{2A}-adrenoceptor (Simmler et al. 2014b) likely contributes to the sympathomimetic effect of 2-AI. Interestingly, 2-AI was a full agonist at the human TAAR1 with similar potency (EC_{50} of 1.5 μM) similar to amphetamine (EC_{50} of 2.8 μM), which also has full-agonist properties (Simmler et al. 2016).

4.5 Conclusions

Amphetamine-like NPS preferentially inhibit DAT and NET and act as releasers. The NPS discussed here show preference for DAT over SERT inhibition, but with lower DAT/SERT ratios than amphetamine. The receptor interaction profiles of amphetamine-like NPS vary and might contribute to drug-specific psychotropic effects and toxicity. MAO inhibition has been shown for 5-IT and suggests particular risk for fatal intoxication.

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References

Adamowicz P, Zuba D, Byrska B (2014) Fatal intoxication with 3-methyl-N-methylcathinone (3-MMC) and 5-(2-aminopropyl)benzofuran (5-APB). Forensic Sci Int 245:126–132. https://doi.org/10.1016/j.forsciint.2014.10.016

Aerts LA, Mallaret M, Rigter H (2000) N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB): its properties and possible risks. Addict Biol 5(3):269–282. https://doi.org/10.1111/j.1369-1600.2000.tb00191.x

Alex KD, Pehek EA (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. Pharmacol Ther 113(2):296–320. https://doi.org/10.1016/j.pharmthera.2006.08.004

Bäckberg M, Beck O, Hulten P, Rosengren-Holmberg J, Helander A (2014) Intoxications of the new psychoactive substance 5-(2-aminopropyl)indole (5-IT): a case series from the Swedish STRIDA project. Clin Toxicol (Phila) 52(6):618–624. https://doi.org/10.3109/15563650.2014.920088

Bauer CT, Banks ML, Blough BE, Negus SS (2013) Use of intracranial self-stimulation to evaluate abuse-related and abuse-limiting effects of monoamine releasers in rats. Br J Pharmacol 168(4):850–862. https://doi.org/10.1111/j.1476-5381.2012.02214.x

Baumann MH, Wang X, Rothman RB (2007) 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. Psychopharmacology 189(4):407–424. https://doi.org/10.1007/s00213-006-0322-6

Baumann MH, Clark RD, Woolverton WL, Wee S, Blough BE, Rothman RB (2011) In vivo effects of amphetamine analogs reveal evidence for serotonergic inhibition of mesolimbic dopamine transmission in the rat. J Pharmacol Exp Ther 337(1):218–225. https://doi.org/10.1124/jpet.110.176271

Baumann MH, Ayestas MA Jr, Partilla JS, Sink JR, Shulgin AT, Daley PF, Brandt SD, Rothman RB, Ruoho AE, Cozzi NV (2012) The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. Neuropsychopharmacology 37(5):1192–1203. https://doi.org/10.1038/npp.2011.304

Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF, Holy M, Rothman RB, Goldberg SR, Lupica CR, Sitte HH, Brandt SD, Tella SR, Cozzi NV, Schindler CW (2013) Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive 'bath salts' products. Neuropsychopharmacology 38(4):552–562. https://doi.org/10.1038/npp.2012.204

Bershad AK, Miller MA, Baggott MJ, de Wit H (2016) The effects of MDMA on socio-emotional processing: does MDMA differ from other stimulants? J Psychopharmacol 30:1248–1258. https://doi.org/10.1177/0269881116663120

Blakely RD, Defelice LJ, Galli A (2005) Biogenic amine neurotransmitter transporters: just when you thought you knew them. Physiology 20:225–231. https://doi.org/10.1152/physiol.00013.2005

Blanckaert P, van Amsterdam J, Brunt T, van den Berg J, Van Durme F, Maudens K, van Bussel J (2013) 4-Methyl-amphetamine: a health threat for recreational amphetamine users. J Psychopharmacol 27(9):817–822. https://doi.org/10.1177/0269881113487950

Bonano JS, Glennon RA, De Felice LJ, Banks ML, Negus SS (2014) Abuse-related and abuse-limiting effects of methcathinone and the synthetic "bath salts" cathinone analogs methylenedioxypyrovalerone (MDPV), methylone and mephedrone on intracranial self-stimulation in rats. Psychopharmacology 231(1):199–207. https://doi.org/10.1007/s00213-013-3223-5

Bradaia A, Trube G, Stalder H, Norcross RD, Ozmen L, Wettstein JG, Pinard A, Buchy D, Gassmann M, Hoener MC, Bettler B (2009) The selective antagonist EPPTB reveals TAAR1-mediated regulatory mechanisms in dopaminergic neurons of the mesolimbic system. Proc Natl Acad Sci U S A 106(47):20081–20086. https://doi.org/10.1073/pnas.0906522106

Brandt SD, Braithwaite RA, Evans-Brown M, Kicman AT (2013) Aminoindane analogues. In: Dargan PI, Wood DM (eds) Novel psychoactive substances: classification, pharmacology and toxicology. Elsevier, London, pp 261–283. https://doi.org/10.1016/B978-0-12-415816-0.00011-0

Bunzow JR, Sonders MS, Arttamangkul S, Harrison LM, Zhang G, Quigley DI, Darland T, Suchland KL, Pasumamula S, Kennedy JL, Olson SB, Magenis RE, Amara SG, Grandy DK (2001) Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. Mol Pharmacol 60(6):1181–1188. https://doi.org/10.1124/mol.60.6.1181

Carmo H, Remiao F, Carvalho F, Fernandes E, de Boer D, dos Reys LA, de Lourdes Bastos M (2003) 4-Methylthioamphetamine-induced hyperthermia in mice: influence of serotonergic and catecholaminergic pathways. Toxicol Appl Pharmacol 190(3):262–271

Colado MI, Granados R, O'Shea E, Esteban B, Green AR (1999) The acute effect in rats of 3,4-methylenedioxyethamphetamine (MDEA, "eve") on body temperature and long term degeneration of 5-HT neurones in brain: a comparison with MDMA ("ecstasy"). Pharmacol Toxicol 84(6):261–266

Cole JC, Sumnall HR (2003) Altered states: the clinical effects of ecstasy. Pharmacol Ther 98(1):35–58

Corkery JM, Elliott S, Schifano F, Corazza O, Ghodse AH (2013) MDAI (5,6-methylenedioxy-2-aminoindane; 6,7-dihydro-5H-cyclopenta[f][1,3]benzodioxol-6-amine; 'sparkle'; 'mindy') toxicity: a brief overview and update. Hum Psychopharmacol 28(4):345–355. https://doi.org/10.1002/hup.2298

Cruickshank CC, Dyer KR (2009) A review of the clinical pharmacology of methamphetamine. Addiction 104(7):1085–1099. https://doi.org/10.1111/j.1360-0443.2009.02564.x

Daw ND, Kakade S, Dayan P (2002) Opponent interactions between serotonin and dopamine. Neural Netw 15(4–6):603–616. https://doi.org/10.1016/S0893-6080(02)00052-7

Dawson P, Opacka-Juffry J, Moffatt JD, Daniju Y, Dutta N, Ramsey J, Davidson C (2014) The effects of benzofury (5-APB) on the dopamine transporter and 5-HT2-dependent vasoconstriction in the rat. Prog Neuro-Psychopharmacol Biol Psychiatry 48:57–63. https://doi.org/10.1016/j.pnpbp.2013.08.013

Di Cara B, Maggio R, Aloisi G, Rivet JM, Lundius EG, Yoshitake T, Svenningsson P, Brocco M, Gobert A, De Groote L, Cistarelli L, Veiga S, De Montrion C, Rodriguez M, Galizzi JP, Lockhart BP, Coge F, Boutin JA, Vayer P, Verdouw PM, Groenink L, Millan MJ (2011) Genetic deletion of trace amine 1 receptors reveals their role in auto-inhibiting the actions of ecstasy (MDMA). J Neurosci 31(47):16928–16940. https://doi.org/10.1523/JNEUROSCI.2502-11.2011

Dolder PC, Strajhar P, Vizeli P, Hammann F, Odermatt A, Liechti ME (2017) Pharmacokinetics and pharmacodynamics of lisdexamfetamine compared with D-amphetamine in healthy subjects. Front Pharmacol 8:617. https://doi.org/10.3389/fphar.2017.00617

Dukat M, Young R, Glennon RA (2002) Effect of PMA optical isomers and 4-MTA in PMMA-trained rats. Pharmacol Biochem Behav 72(1–2):299–305. https://doi.org/10.1016/S0091-3057(01)00776-6

Elliott S, Evans J (2014) A 3-year review of new psychoactive substances in casework. Forensic Sci Int 243:55–60. https://doi.org/10.1016/j.forsciint.2014.04.017

EMCDDA (2014) Report on the risk assessment of 5-(2-aminopropyl)indole in the framework of the Council Decision on new psychoactive substances, risk assessment. Publications Office of the European Union, Luxembourg. https://doi.org/10.2810/21296

EMCDDA (2016) European drug report 2016. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Luxembourg. https://doi.org/10.2810/084165

Eshleman AJ, Wolfrum KM, Reed JF, Kim SO, Swanson T, Johnson RA, Janowsky A (2017) Structure-activity relationships of substituted cathinones, with transporter binding, uptake, and release. J Pharmacol Exp Ther 360(1):33–47. https://doi.org/10.1124/jpet.116.236349

Gannon BM, Sulima A, Rice KC, Collins GT (2018) Inhibition of cocaine and 3,4-methylenedioxypyrovalerone (MDPV) self-administration by lorcaserin is mediated by 5-HT2C receptors in rats. J Pharmacol Exp Ther 364(2):359–366. https://doi.org/10.1124/jpet.117.246082

Gatch MB, Dolan SB, Forster MJ (2016) Locomotor, discriminative stimulus, and place conditioning effects of MDAI in rodents. Behav Pharmacol 27(6):497–505. https://doi.org/10.1097/FBP.0000000000000237

Gillman PK (2005) Monoamine oxidase inhibitors, opioid analgesics and serotonin toxicity. Br J Anaesth 95(4):434–441. https://doi.org/10.1093/bja/aei210

Glennon RA, Young R, Hauck AE, McKenney JD (1984) Structure-activity studies on amphetamine analogs using drug discrimination methodology. Pharmacol Biochem Behav 21(6):895–901

Glennon RA, Yousif M, Patrick G (1988) Stimulus properties of 1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDA) analogs. Pharmacol Biochem Behav 29(3):443–449

Glennon RA, Young R, Dukat M, Chang-Fong J, El-Zahabi M (2007) N-methyl-1-(4-methoxyphenyl)-2-aminopropane (PMMA) and N-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDMA) produce non-identical discriminative stimuli in rats. Pharmacol Biochem Behav 86(3):477–484. https://doi.org/10.1016/j.pbb.2007.01.007

Harro J (2015) Neuropsychiatric adverse effects of amphetamine and methamphetamine. Int Rev Neurobiol 120:179–204. https://doi.org/10.1016/bs.irn.2015.02.004

Hartung TK, Schofield E, Short AI, Parr MJ, Henry JA (2002) Hyponatraemic states following 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') ingestion. QJM 95(7):431–437

Heal DJ, Smith SL, Gosden J, Nutt DJ (2013) Amphetamine, past and present – a pharmacological and clinical perspective. J Psychopharmacol 27(6):479–496. https://doi.org/10.1177/0269881113482532

Herraiz T, Brandt SD (2014) 5-(2-Aminopropyl)indole (5-IT): a psychoactive substance used for recreational purposes is an inhibitor of human monoamine oxidase (MAO). Drug Test Anal 6(7–8):607–613. https://doi.org/10.1002/dta.1530

Hill SL, Thomas SH (2011) Clinical toxicology of newer recreational drugs. Clin Toxicol (Phila) 49(8):705–719. https://doi.org/10.3109/15563650.2011.615318

Holden R, Jackson MA (1996) Near-fatal hyponatraemic coma due to vasopressin over-secretion after "ecstasy" (3,4-MDMA). Lancet 347(9007):1052

Hondebrink L, Nugteren-van Lonkhuyzen JJ, Rietjens SJ, Brunt TM, Venhuis B, Soerdjbalie-Maikoe V, Smink BE, van Riel A, de Vries I (2017) Fatalities, cerebral hemorrhage, and severe cardiovascular toxicity after exposure to the new psychoactive substance 4-fluoroamphetamine: a prospective cohort study. Ann Emerg Med. https://doi.org/10.1016/j.annemergmed.2017.07.482

Horn AS, Snyder SH (1972) Steric requirements for catecholamine uptake by rat brain synaptosomes: studies with rigid analogs of amphetamine. J Pharmacol Exp Ther 180(3):523–530

Hysek CM, Vollenweider FX, Liechti ME (2010) Effects of a beta-blocker on the cardiovascular response to MDMA (ecstasy). Emerg Med J 27(8):586–589. https://doi.org/10.1136/emj.2009.079905

Hysek CM, Simmler LD, Ineichen M, Grouzmann E, Hoener MC, Brenneisen R, Huwyler J, Liechti ME (2011) The norepinephrine transporter inhibitor reboxetine reduces stimulant effects of MDMA ("ecstasy") in humans. Clin Pharmacol Ther 90(2):246–255. https://doi.org/10.1038/clpt.2011.78

Hysek CM, Schmid Y, Rickli A, Simmler LD, Donzelli M, Grouzmann E, Liechti ME (2012a) Carvedilol inhibits the cardiostimulant and thermogenic effects of MDMA in humans. Br J Pharmacol 166(8):2277–2288. https://doi.org/10.1111/j.1476-5381.2012.01936.x

Hysek CM, Brugger R, Simmler LD, Bruggisser M, Donzelli M, Grouzmann E, Hoener MC, Liechti ME (2012b) Effects of the alpha(2)-adrenergic agonist clonidine on the pharmacodynamics and pharmacokinetics of 3,4-methylenedioxymethamphetamine in healthy volunteers. J Pharmacol Exp Ther 340(2):286–294. https://doi.org/10.1124/jpet.111.188425

Hysek CM, Simmler LD, Nicola VG, Vischer N, Donzelli M, Krahenbuhl S, Grouzmann E, Huwyler J, Hoener MC, Liechti ME (2012c) Duloxetine inhibits effects of MDMA ("ecstasy") in vitro and in humans in a randomized placebo-controlled laboratory study. PLoS One 7(5): e36476. https://doi.org/10.1371/journal.pone.0036476

Hysek CM, Schmid Y, Simmler LD, Domes G, Heinrichs M, Eisenegger C, Preller KH, Quednow BB, Liechti ME (2014a) MDMA enhances emotional empathy and prosocial behavior. Soc Cogn Affect Neurosci 9(11):1645–1652. https://doi.org/10.1093/scan/nst161

Hysek CM, Simmler LD, Schillinger N, Meyer N, Schmid Y, Donzelli M, Grouzmann E, Liechti ME (2014b) Pharmacokinetic and pharmacodynamic effects of methylphenidate and MDMA administered alone or in combination. Int J Neuropsychopharmacol 17(3):371–381. https://doi.org/10.1017/S1461145713001132

Iversen L, Gibbons S, Treble R, Setola V, Huang XP, Roth BL (2013) Neurochemical profiles of some novel psychoactive substances. Eur J Pharmacol 700(1–3):147–151. https://doi.org/10.1016/j.ejphar.2012.12.006

Johnson MP, Conarty PF, Nichols DE (1991) [3H]monoamine releasing and uptake inhibition properties of 3,4-methylenedioxymethamphetamine and p-chloroamphetamine analogues. Eur J Pharmacol 200(1):9–16

Kehr J, Ichinose F, Yoshitake S, Goiny M, Sievertsson T, Nyberg F, Yoshitake T (2011) Mephedrone, compared to MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and serotonin levels in nucleus accumbens of awake rats. Br J Pharmacol. https://doi.org/10.1111/j.1476-5381.2011.01499.x

Koob GF, Volkow ND (2016) Neurobiology of addiction: a neurocircuitry analysis. Lancet Psychiatry 3(8):760–773. https://doi.org/10.1016/S2215-0366(16)00104-8

Liechti ME (2014) Effects of MDMA on body temperature in humans. Temperature (Austin) 1(3):192–200. https://doi.org/10.4161/23328940.2014.955433

Liechti M (2015) Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signaling. Swiss Med Wkly 145:w14043. https://doi.org/10.4414/smw.2015.14043

Liechti ME, Saur MR, Gamma A, Hell D, Vollenweider FX (2000) Psychological and physiological effects of MDMA ("ecstasy") after pretreatment with the 5-HT(2) antagonist ketanserin in healthy humans. Neuropsychopharmacology 23(4):396–404. https://doi.org/10.1016/S0893-133X(00)00126-3

Liechti ME, Gamma A, Vollenweider FX (2001) Gender differences in the subjective effects of MDMA. Psychopharmacology 154(2):161–168

Lindemann L, Meyer CA, Jeanneau K, Bradaia A, Ozmen L, Bluethmann H, Bettler B, Wettstein JG, Borroni E, Moreau JL, Hoener MC (2008) Trace amine-associated receptor 1 modulates dopaminergic activity. J Pharmacol Exp Ther 324(3):948–956. https://doi.org/10.1124/jpet.107.132647

Linsen F, Koning RP, van Laar M, Niesink RJ, Koeter MW, Brunt TM (2015) 4-Fluoroamphetamine in the Netherlands: more than a one-night stand. Addiction 110(7):1138–1143. https://doi.org/10.1111/add.12932

Luethi D, Kolaczynska KE, Docci L, Krahenbuhl S, Hoener MC, Liechti ME (2017) Pharmacological profile of mephedrone analogs and related new psychoactive substances. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2017.07.026

Lurie Y, Gopher A, Lavon O, Almog S, Sulimani L, Bentur Y (2012) Severe paramethoxymethamphetamine (PMMA) and paramethoxyamphetamine (PMA) outbreak in Israel. Clin Toxicol (Phila) 50(1):39–43. https://doi.org/10.3109/15563650.2011.635148

Luscher C, Malenka RC (2011) Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. Neuron 69(4):650–663. https://doi.org/10.1016/j.neuron.2011.01.017

Marusich JA, Antonazzo KR, Blough BE, Brandt SD, Kavanagh PV, Partilla JS, Baumann MH (2016) The new psychoactive substances 5-(2-aminopropyl)indole (5-IT) and 6-(2-aminopropyl)indole (6-IT) interact with monoamine transporters in brain tissue. Neuropharmacology 101:68–75. https://doi.org/10.1016/j.neuropharm.2015.09.004

Mash DC, Duque L, Pablo J, Qin Y, Adi N, Hearn WL, Hyma BA, Karch SB, Druid H, Wetli CV (2009) Brain biomarkers for identifying excited delirium as a cause of sudden death. Forensic Sci Int 190(1–3):e13–e19. https://doi.org/10.1016/j.forsciint.2009.05.012

Matsumoto T, Maeno Y, Kato H, Seko-Nakamura Y, Monma-Ohtaki J, Ishiba A, Nagao M, Aoki Y (2014) 5-Hydroxytryptamine- and dopamine-releasing effects of ring-substituted amphetamines on rat brain: a comparative study using in vivo microdialysis. Eur Neuropsychopharmacol 24(8):1362–1370. https://doi.org/10.1016/j.euroneuro.2014.04.009

McIntyre IM, Gary RD, Trochta A, Stolberg S, Stabley R (2015) Acute 5-(2-aminopropyl) benzofuran (5-APB) intoxication and fatality: a case report with postmortem concentrations. J Anal Toxicol 39(2):156–159. https://doi.org/10.1093/jat/bku131

Monte AP, Marona-Lewicka D, Cozzi NV, Nichols DE (1993) Synthesis and pharmacological examination of benzofuran, indan, and tetralin analogues of 3,4-(methylenedioxy)amphetamine. J Med Chem 36(23):3700–3706

Mrongovius RI, Bolt AG, Hellyer RO (1978) Comparison of the anorectic and motor activity effects of some aminoindanes, 2-aminotetralin and amphetamine in the rat. Clin Exp Pharmacol Physiol 5(6):635–640

Nichols DE (1986) Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens. J Psychoactive Drugs 18(4):305–313. https://doi.org/10.1080/02791072.1986.10472362

Nichols DE (2004) Hallucinogens. Pharmacol Ther 101(2):131–181. https://doi.org/10.1016/j.pharmthera.2003.11.002

Nichols DE (2017) Chemistry and structure-activity relationships of psychedelics. Curr Top Behav Neurosci. https://doi.org/10.1007/7854_2017_475

Nichols DE, Brewster WK, Johnson MP, Oberlender R, Riggs RM (1990) Nonneurotoxic tetralin and indan analogues of 3,4-(methylenedioxy)amphetamine (MDA). J Med Chem 33(2):703–710

Nichols DE, Johnson MP, Oberlender R (1991) 5-Iodo-2-aminoindan, a nonneurotoxic analogue of p-iodoamphetamine. Pharmacol Biochem Behav 38(1):135–139

Palenicek T, Lhotkova E, Zidkova M, Balikova M, Kuchar M, Himl M, Miksatkova P, Cegan M, Vales K, Tyls F, Horsley RR (2016) Emerging toxicity of 5,6-methylenedioxy-2-aminoindane (MDAI): pharmacokinetics, behaviour, thermoregulation and LD50 in rats. Prog Neuro-Psychopharmacol Biol Psychiatry 69:49–59. https://doi.org/10.1016/j.pnpbp.2016.04.004

Pascoli V, Terrier J, Hiver A, Luscher C (2015) Sufficiency of mesolimbic dopamine neuron stimulation for the progression to addiction. Neuron. https://doi.org/10.1016/j.neuron.2015.10.017

Pei Y, Lee J, Leo D, Gainetdinov RR, Hoener MC, Canales JJ (2014) Activation of the trace amine-associated receptor 1 prevents relapse to cocaine seeking. Neuropsychopharmacology 39(10):2299–2308. https://doi.org/10.1038/npp.2014.88

Pei Y, Mortas P, Hoener MC, Canales JJ (2015) Selective activation of the trace amine-associated receptor 1 decreases cocaine's reinforcing efficacy and prevents cocaine-induced changes in brain reward thresholds. Prog Neuro-Psychopharmacol Biol Psychiatry 63:70–75. https://doi.org/10.1016/j.pnpbp.2015.05.014

Pinterova N, Horsley RR, Palenicek T (2017) Synthetic aminoindanes: a summary of existing knowledge. Front Psych 8:236. https://doi.org/10.3389/fpsyt.2017.00236

Prosser JM, Nelson LS (2012) The toxicology of bath salts: a review of synthetic cathinones. J Med Toxicol 8(1):33–42. https://doi.org/10.1007/s13181-011-0193-z

Rickli A, Hoener MC, Liechti ME (2015a) Monoamine transporter and receptor interaction profiles of novel psychoactive substances: para-halogenated amphetamines and pyrovalerone cathinones. Eur Neuropsychopharmacol 25(3):365–376. https://doi.org/10.1016/j.euroneuro.2014.12.012

Rickli A, Kopf S, Hoener MC, Liechti ME (2015b) Pharmacological profile of novel psychoactive benzofurans. Br J Pharmacol 172(13):3412–3425. https://doi.org/10.1111/bph.13128

Rickli A, Luethi D, Reinisch J, Buchy D, Hoener MC, Liechti ME (2015c) Receptor interaction profiles of novel N-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). Neuropharmacology 99:546–553. https://doi.org/10.1016/j.neuropharm.2015.08.034

Roth BL (2007) Drugs and valvular heart disease. N Engl J Med 356(1):6–9. https://doi.org/10.1056/NEJMp068265

Rothman RB, Baumann MH (2006) Balance between dopamine and serotonin release modulates behavioral effects of amphetamine-type drugs. Ann N Y Acad Sci 1074:245–260. https://doi.org/10.1196/annals.1369.064

Sainsbury PD, Kicman AT, Archer RP, King LA, Braithwaite RA (2011) Aminoindanes – the next wave of 'legal highs'? Drug Test Anal 3(7–8):479–482. https://doi.org/10.1002/dta.318

Schindler CW, Thorndike EB, Goldberg SR, Lehner KR, Cozzi NV, Brandt SD, Baumann MH (2016) Reinforcing and neurochemical effects of the "bath salts" constituents 3,4-methylenedioxypyrovalerone (MDPV) and 3,4-methylenedioxy-N-methylcathinone (methylone) in male rats. Psychopharmacology 233(10):1981–1990. https://doi.org/10.1007/s00213-015-4057-0

Schmid Y, Hysek CM, Simmler LD, Crockett MJ, Quednow BB, Liechti ME (2014) Differential effects of MDMA and methylphenidate on social cognition. J Psychopharmacol 28(9):847–856. https://doi.org/10.1177/0269881114542454

Scorza C, Silveira R, Nichols DE, Reyes-Parada M (1999) Effects of 5-HT-releasing agents on the extracellullar hippocampal 5-HT of rats. Implications for the development of novel antidepressants with a short onset of action. Neuropharmacology 38(7):1055–1061

Simmler LD, Hysek CM, Liechti ME (2011) Sex differences in the effects of MDMA (ecstasy) on plasma copeptin in healthy subjects. J Clin Endocrinol Metab 96(9):2844–2850. https://doi.org/10.1210/jc.2011-1143

Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu LH, Huwyler J, Chaboz S, Hoener MC, Liechti ME (2013) Pharmacological characterization of designer cathinones in vitro. Br J Pharmacol 168(2):458–470. https://doi.org/10.1111/j.1476-5381.2012.02145.x

Simmler LD, Rickli A, Hoener MC, Liechti ME (2014a) Monoamine transporter and receptor interaction profiles of a new series of designer cathinones. Neuropharmacology 79:152–160. https://doi.org/10.1016/j.neuropharm.2013.11.008

Simmler LD, Rickli A, Schramm Y, Hoener MC, Liechti ME (2014b) Pharmacological profiles of aminoindanes, piperazines, and pipradrol derivatives. Biochem Pharmacol 88(2):237–244. https://doi.org/10.1016/j.bcp.2014.01.024

Simmler LD, Buchy D, Chaboz S, Hoener MC, Liechti ME (2016) In vitro characterization of psychoactive substances at rat, mouse, and human trace amine-associated receptor 1. J Pharmacol Exp Ther 357(1):134–144. https://doi.org/10.1124/jpet.115.229765

Simmler LD, Anacker AMJ, Levin MH, Vaswani NM, Gresch PJ, Nackenoff AG, Anastasio NC, Stutz SJ, Cunningham KA, Wang J, Zhang B, Henry LK, Stewart A, Veenstra-VanderWeele J, Blakely RD (2017) Blockade of the 5-HT transporter contributes to the behavioural, neuronal and molecular effects of cocaine. Br J Pharmacol 174(16):2716–2738. https://doi.org/10.1111/bph.13899

Sprague JE, Brutcher RE, Mills EM, Caden D, Rusyniak DE (2004) Attenuation of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy)-induced rhabdomyolysis with alpha1- plus beta3-adrenoreceptor antagonists. Br J Pharmacol 142(4):667–670. https://doi.org/10.1038/sj.bjp.0705823

Sulzer D (2011) How addictive drugs disrupt presynaptic dopamine neurotransmission. Neuron 69(4):628–649. https://doi.org/10.1016/j.neuron.2011.02.010

Suyama JA, Sakloth F, Kolanos R, Glennon RA, Lazenka MF, Negus SS, Banks ML (2016) Abuse-related neurochemical effects of para-substituted methcathinone analogs in rats: microdialysis studies of nucleus accumbens dopamine and serotonin. J Pharmacol Exp Ther 356(1):182–190. https://doi.org/10.1124/jpet.115.229559

Tessel RE, Rutledge CO (1976) Specificity of release of biogenic amines from isolated rat brain tissue as a function of the meta substituent of N-ethylamphetamine derivatives. J Pharmacol Exp Ther 197(2):253–262

Tessel RE, Woods JH (1975) Fenfluramine and N-ethyl amphetamine: comparison of the reinforcing and rate-decreasing actions in the rhesus monkey. Psychopharmacologia 43(3):239–244

Tessel RE, Woods JH, Counsell RE, Lu M (1975) Structure-activity relationships between meta-substituted N-ethylamphetamines and locomotor activity in mice. J Pharmacol Exp Ther 192(2):310–318

Torres GE, Gainetdinov RR, Caron MG (2003) Plasma membrane monoamine transporters: structure, regulation and function. Nat Rev Neurosci 4(1):13–25. https://doi.org/10.1038/nrn1008

Verrico CD, Miller GM, Madras BK (2007) MDMA (ecstasy) and human dopamine, norepinephrine, and serotonin transporters: implications for MDMA-induced neurotoxicity and treatment. Psychopharmacology 189(4):489–503. https://doi.org/10.1007/s00213-005-0174-5

Vevelstad M, Oiestad EL, Middelkoop G, Hasvold I, Lilleng P, Delaveris GJ, Eggen T, Morland J, Arnestad M (2012) The PMMA epidemic in Norway: comparison of fatal and non-fatal intoxications. Forensic Sci Int 219(1–3):151–157. https://doi.org/10.1016/j.forsciint.2011.12.014

Vizeli P, Liechti ME (2017) Safety pharmacology of acute MDMA administration in healthy subjects. J Psychopharmacol 31(5):576–588. https://doi.org/10.1177/0269881117691569

Wee S, Anderson KG, Baumann MH, Rothman RB, Blough BE, Woolverton WL (2005) Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. J Pharmacol Exp Ther 313(2):848–854. https://doi.org/10.1124/jpet.104.080101

Welter-Luedeke J, Maurer HH (2016) New psychoactive substances: chemistry, pharmacology, metabolism, and detectability of amphetamine derivatives with modified ring systems. Ther Drug Monit 38(1):4–11. https://doi.org/10.1097/FTD.0000000000000240

Wijers CH, van Litsenburg RT, Hondebrink L, Niesink RJ, Croes EA (2017) Acute toxic effects related to 4-fluoroamphetamine. Lancet 389(10069):600. https://doi.org/10.1016/S0140-6736(17)30281-7

Woolverton WL, English JA (1997) Effects of some phenylethylamines in rhesus monkeys trained to discriminate (+)-amphetamine from saline. Drug Alcohol Depend 44(2–3):79–85

Zwartsen A, Verboven AHA, van Kleef R, Wijnolts FMJ, Westerink RHS, Hondebrink L (2017) Measuring inhibition of monoamine reuptake transporters by new psychoactive substances (NPS) in real-time using a high-throughput, fluorescence-based assay. Toxicol In Vitro 45(Pt 1):60–71. https://doi.org/10.1016/j.tiv.2017.05.010

The Chemistry and Pharmacology of Synthetic Cannabinoid Receptor Agonists as New Psychoactive Substances: Origins

Samuel D. Banister and Mark Connor

Contents

Abstract

Synthetic cannabinoid receptor agonists (SCRAs) have proliferated as new psychoactive substances (NPS) over the past decade. Relative to other classes of NPS, SCRAs are structurally heterogeneous; however, most SCRAs act as potent, high-efficacy agonists of cannabinoid type 1 and type 2 receptors (CB_1 and CB_2, respectively). Characterization of the pharmacology and toxicology of these substances is hindered by the dynamic nature of the SCRA marketplace. Beyond basic pharmacological profiling at CB_1 and CB_2 receptors, very little is known about the acute or chronic effects of SCRAs. Many of the effects of SCRAs are

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qualitatively similar to those of the Δ^9-tetrahydrocannabinol (Δ^9-THC) found in cannabis. However, unlike Δ^9-THC, SCRAs are frequently associated with serious adverse effects, including cardiotoxicity, nephrotoxicity, and death. This chapter will provide an overview of the structure and function of the primary target for SCRAs, the CB_1 receptor, and survey the structure-activity relationships of the historical SCRAs that served as templates for the earliest generations of NPS.

Keywords
Anandamide · Cannabinoid · CP 55,940 · JWH-018 · NPS · Δ^9-Tetrahydrocannabinol · WIN 55,212-2 · XLR-11

Acronyms of the Discussed New Psychoactive Substances (NPS)

5F-AB-001	(Adamantan-1-yl)[1-(5-fluoropentyl)-1*H*-indol-3-yl]methanone
A-796,260	[1-(2-Morpholin-4-ylethyl)-1*H*-indol-3-yl]-(2,2,3,3-tetramethylcyclopropyl)methanone
AB-001	(Adamantan-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone
AB-005	{1-[(1-Methylpiperidin-2-yl)methyl]-1*H*-indol-3-yl}(2,2,3,3-tetramethylcyclopropyl)methanone
AB-FUBINACA	(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide
2-AG	2-Arachidonoylglycerol
AEA	Arachidonylethanolamide
AM-679	(2-Iodophenyl)(1-pentyl-1*H*-indol-3-yl)methanone
AM-1241	(2-Iodo-5-nitrophenyl){1-[(1-methylpiperidin-2-yl)methyl]-1*H*-indol-3-yl}methanone
AM-1248	[1-(1-Methylpiperidin-2-yl)-1*H*-indol-3-yl](adamant-1-yl)methanone
AM-2201	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](naphthalen-1-yl)methanone
AM-2232	[1-(4-Cyanobutyl)-1*H*-indol-3-yl](naphthalen-1-yl)methanone
AM-2233	(2-Iodophenyl){1-[(1-methyl-2-piperidinyl)methyl]-1*H*-indol-3-yl}methanone
BB-22	8-Quinolinyl-1-(cyclohexylmethyl)-1*H*-indole-3-carboxylate
CBD	Cannabidiol
CBN	Cannabinol
CBND	Cannabinodiol
CCH	Cannabicyclohexanol
CP 47,497	2-[(1*R*,3*S*)-3-Hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol
CP 47,497-C8	2-[(1*R*,3*S*)-3-Hydroxycyclohexyl]-5-(2-methylnonan-2-yl)phenol
CP 55,940	2-[(1*R*,2*R*,5*R*)-5-Hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol

CPE	Cannabipiperidiethanone
DMHP	Dimethylheptylpyran
EAM-2201	(4-Ethylnaphthalen-1-yl)[1-(5-fluoropentyl)indol-3-yl] methanone
HHC	9-nor-9β-Hydroxyhexahydrocannabinol
HU-210	(6a*R*,10a*R*)-9-(Hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[*c*] chromen-1-ol
JWH-018	Naphthalen-1-yl(1-pentyl-1*H*-indol-3-yl)methanone
JWH-019	(1-Hexyl-1*H*-indol-3-yl)(naphthalen-1-yl)methanone
JWH-020	(1-Heptyl-1*H*-indol-3-yl)(naphthalen-1-yl)methanone
JWH-030	(1-Hexylpyrrol-3-yl)-naphthalen-1-ylmethanone
JWH-073	(1-Butyl-1*H*-indol-3-yl)(naphthalen-1-yl)methanone
JWH-081	(4-Methoxynaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl) methanone
JWH-122	(4-Methylnaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone
JWH-145	Naphthalen-1-yl(1-pentyl-5-phenyl-1*H*-pyrrol-3-yl)methanone
JWH-147	(1-Hexyl-5-phenyl-1*H*-pyrrol-3-yl)-naphthalen-1-ylmethanone
JWH-182	(1-Pentyl-1*H*-indol-3-yl)(4-propylnaphthalen-1-yl)methanone
JWH-203	2-(2-Chlorophenyl)-1-(2-methyl-1-pentyl-1*H*-indol-3-yl) ethanone
JWH-210	(4-Ethyl-1-naphthalenyl)(1-pentyl-1*H*-indol-3-yl)-methanone
JWH-250	2-(2-Methoxyphenyl)-1-(1-pentyl-1*H*-indol-3-yl)ethanone
JWH-251	2-(3-Methylphenyl)-1-(1-pentyl-1*H*-indol-3-yl)ethanone
JWH-307	[5-(2-Fluorophenyl)-1-pentyl-1*H*-pyrrol-3-yl](naphthalene-1-yl)methanone
JWH-398	(4-Chloronaphthalen-1-yl)(1-pentyl-1*H*-indole-3-yl)methanone
JWH-412	(4-Fluoronaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone
MAM-2201	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](4-methylnaphthalen-1-yl) methanone
RCS-2	(2-Methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone
RCS-4	(4-Methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone
RCS-8	1-[1-(2-Cyclohexylethyl)-1*H*-indol-3-yl]-2-(2-methoxyphenyl) ethanone
Δ^8-THC	(−)-*trans*-Δ^8-Tetrahydrocannabinol
Δ^9-THC	(−)-*trans*-Δ^9-Tetrahydrocannabinol
UR-144	(1-Pentyl-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl) methanone
WIN 55,212-2	(*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone
XLR-11	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone
XLR-12	(2,2,3,3-Tetramethylcyclopropyl)[1-(4,4,4-trifluorobutyl)-1*H*-indol-3-yl]methanone

1 Introduction

Since the first conclusive identification of synthetic cannabinoid receptor agonists (SCRAs) as new psychoactive substances (NPS) in 2008, more than 230 SCRA NPS have been reported in 106 countries in all regions of the world (UNODC 2017). SCRA raw materials are most commonly manufactured in China, imported to the country of sale by organized crime groups or entrepreneurs, and applied to dried plant matter for use as smoking blends with superficial similarity to cannabis. The first SCRA NPS were detected in herbal blends packaged in foil sachets and disingenuously marketed as "incense," "potpourri," "air freshener," and "not for human consumption." The first confirmed SCRA NPS product was branded "Spice," a reference to the fictional narcotic drug of the same name in Frank Herbert's science fiction novel Dune. Many other SCRA NPS products have appeared since, branded with names like K2 and Black Mamba, although Spice has remained a generic term for SCRA NPS. Like the branded packaging, the SCRA constituents of these products change rapidly in response to legislative controls, with little consistency between products (Dresen et al. 2010). Less commonly, SCRAs are sold as bulk powders of high purity, as liquid formulations for vaporization in electronic cigarettes, blended into a dough-like substance as "fake hash," and in edible products

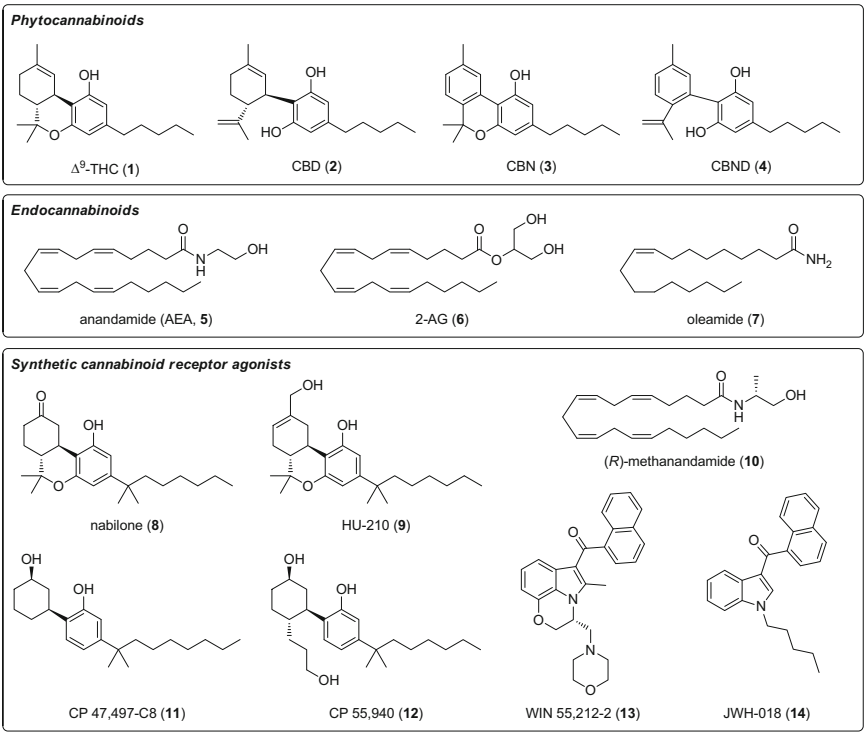


Fig. 1 Representative phyto-, endo-, and synthetic cannabinoid chemotypes

such as candy and baked goods (Peace et al. 2017; Obafemi et al. 2015; Angerer et al. 2015; EMCDDA 2017).

SCRAs represent the largest and most structurally diverse class of NPS. However, like the principal psychoactive component of cannabis, (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**, Fig. 1), all are intended to elicit psychoactive effects by activating cannabinoid type 1 receptors in the brain. Unlike cannabis, SCRAs are frequently associated with serious adverse effects, including death (Trecki et al. 2015). The differing toxicological profiles of SCRAs and Δ^9-THC are attributed to differences of chemical structure, metabolism, and pharmacology. The first generation of SCRA NPS were repurposed compounds sourced directly from published academic research and patents, but by 2010 new structures with no precedent in the scientific literature were appearing.

The proliferation of SCRAs and other NPS in the Internet age can be viewed as a consequence of (1) the democratization of medicinal chemistry knowledge, (2) inexpensive and accessible chemical manufacturing by contract research organizations (CROs) in China and elsewhere, (3) the response of retailers to constantly changing legislation domestically and internationally, and (4) an inability for traditional legislative procedures to keep pace with a sudden and dramatic increase in the number of recreational drugs not under international control. The chemical complexity and dynamic nature of the SCRA market presents an ongoing challenge for medical professionals, law enforcement officers, and policymakers across the globe. The current chapter will describe the chemistry and pharmacology of historical SCRAs developed prior to their discovery as NPS, and the subsequent chapter will review the structural evolution of emergent SCRAs identified in the 2010s.

2 Chemical Classification of Synthetic Cannabinoid Receptor Agonists

The term *cannabinoid* is broad and refers to a specific class of compounds produced by the cannabis plant (phytocannabinoids produced by *Cannabis sativa* and *Cannabis indica*), as well as endogenous and exogenous ligands that interact with cannabinoid type 1 and type 2 receptors (CB_1 and CB_2, respectively). Endogenous cannabinoids (endocannabinoids) are lipid-signaling molecules structurally distinct from phytocannabinoids. Synthetic cannabinoid receptor agonists comprise several distinct chemotypes, some of which are structurally related to phyto- and endocannabinoids (Fig. 1). Several structurally unique natural products that act as CB_1 and CB_2 agonists in vitro have been identified in plants other than cannabis; however, none have been identified as NPS (Gertsch et al. 2010; Russo 2016).

The cannabis plant has been cultivated by humans for thousands of years as a source of textiles, food, and herbal medicine, and the use of cannabis flowers and leaves as recreational substances has been reported since antiquity (Zuardi 2006). Cannabis contains more than 560 chemical constituents, of which a class of approximately 120 terpenoids are unique to cannabis and are named phytocannabinoids

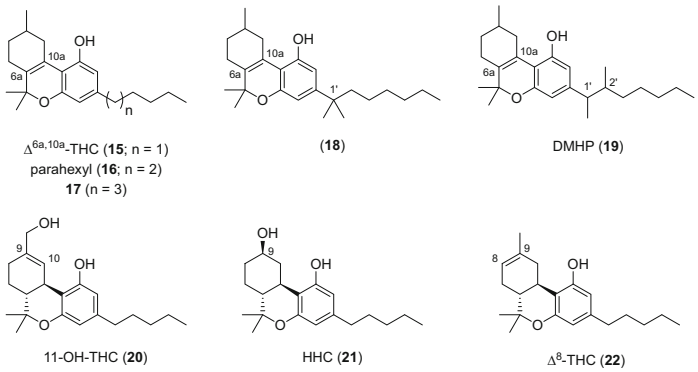


Fig. 2 Classical cannabinoids and phytocannabinoid analogs

(ElSohly et al. 2017; Turner et al. 2017). The most abundant phytocannabinoids obtained from cannabis are Δ^9-THC and non-psychoactive (−)-cannabidiol (CBD, **2**). Other minor phytocannabinoids, including cannabinol (CBN, **3**) and cannabinodiol (CBND, **4**), are also psychoactive but are found in cannabis at concentrations likely insufficient to contribute to psychoactivity. In addition to being listed in Schedule I, cannabis was placed in the strictest category (Schedule IV) of the United Nations 1961 Single Convention on Narcotic Drug and remains the most widely used illicit drug today.

The structural elucidation of Δ^9-THC as the psychoactive component of cannabis occurred in 1964 (Gaoni and Mechoulam 1964). The mammalian target responsible for the activity of Δ^9-THC, a class A G protein-coupled receptor (GPCR), was cloned from rat brain in 1990 (rCB_1) (Matsuda et al. 1990). Shortly thereafter, human CB_1 (hCB_1) was cloned and demonstrated 97% sequence identity at the amino acid level with rCB_1 (Gérard et al. 1991). An endogenous eicosanoid ligand for CB_1, *N*-arachidonoylethanolamide (AEA, **5**), was isolated from pig brain in 1992 and named anandamide from the Sanskrit word for bliss (Devane et al. 1992). A second cannabinoid receptor subtype (CB_2) was discovered in macrophages in 1993 (Munro et al. 1993) and a second endocannabinoid, 2-arachidonoylglycerol (2-AG, **6**), from canine gut in 1995 (Mechoulam et al. 1995). Oleamide (**7**) is an endogenous ligand that was also shown to act as a selective CB_1 agonist (Leggett et al. 2004). CB_1 and CB_2 are extensively characterized receptors of an endocannabinoid system (ECS) and differ in distribution, ligand selectivity, and function (Howlett et al. 2002; Mackie 2008). Several orphan and recently deorphanized GPCRs have been proposed as putative members of the ECS based on their interaction with cannabinoid ligands; however, these receptors typically show little sequence homology with CB_1 and CB_2 (Pertwee et al. 2010; Morales and Reggio 2017). Other components of the ECS include cellular machinery involved in the biosynthesis, metabolism, and transport of endocannabinoids, and have seldom been targeted by NPS manufacturers (Piomelli 2003; Di Marzo 2008).

Many SCRAs were developed by academic and pharmaceutical laboratories to probe structure-activity relationships (SARs) and develop new therapeutics. The origins of many SCRAs are provided by the alphabetic prefix of their code names. Some common prefixes are CP (Pfizer), WIN (Sterling-Winthrop), HU (Raphael Mechoulam, Hebrew University), JWH (John W. Huffman, Clemson University), and AM (Alexandros Makriyannis, University of Connecticut and later Northeastern University).

Classical cannabinoids are those based on the tricyclic benzopyran scaffold of Δ^9-THC, such as nabilone (**8**) and HU-210 (**9**). Others are analogs of endocannabinoids, like (*R*)-methanandamide (**10**), which possesses greater resistance to amide hydrolysis than anandamide. Nonclassical cannabinoids lack the pyran ring of Δ^9-THC and are typified by CP 47,497-C8 (**11**) and CP 55,940 (**12**), which have become important research tools. Several classical and nonclassical cannabinoids, as well as endocannabinoid analogs, have been detected as SCRA NPS. However, the majority of SCRA NPS belong to the aminoalkylindole (AAI) class, of which WIN 55,212-2 (**13**) and JWH-018 (**14**) are representative examples. Historically, SCRAs showed little discrimination between CB_1 and CB_2, and most SCRA NPS interact with both subtypes, albeit usually with a preference for CB_1.

3 Cannabinoid Type 1 Receptor (CB_1) Structure and Function

CB_1 is abundant in the central and peripheral nervous system (CNS and PNS, respectively), while CB_1 levels in peripheral tissues (such as heart, lung, liver, and reproductive organs) are much lower than those in the brain (Galiègue et al. 1995). In contrast, CB_2 occurs mainly in immune cells, although it is upregulated in microglia under conditions of neuroinflammation. The neuroanatomical distribution of CB_1 in cortex, amygdala, hippocampus, basal ganglia, and cerebellum correlate with the effects of CB_1 agonists on cognition, motivation, memory, motor function, and analgesia (Mackie 2005). CB_1 is one of the most abundant GPCRs in the brain and is present at much higher levels than other GPCRs (Herkenham et al. 1991). Within neurons, CB_1 is often localized in axon terminals where its activation inhibits glutamatergic, γ-aminobutyric acid (GABA)ergic, glycinergic, cholinergic, noradrenergic, and serotonergic neurotransmission via presynaptic mechanisms (Szabo and Schlicker 2005). There is increasing evidence that CB_1 may also be located, post-synaptically, in nonneuronal cell types (e.g., astrocytes) and in intracellular organelles (e.g., mitochondria) (Busquets et al. 2016).

Very recently, X-ray crystal structures of human CB_1 bound to antagonists and agonists were reported, and they will undoubtedly provide insight into SCRA-CB_1 interactions (Hua et al. 2016; Shao et al. 2016; Hua et al. 2017). Like other class A GPCRs, CB_1 possesses seven transmembrane domains (TM1–7), an extracellular N-terminal region, and intracellular domains, including an intracellular C-terminal domain which interacts with a G protein complex. CB_1 also possesses one or more modulatory allosteric binding sites, and several classes of positive and negative allosteric modulators have been reported. CB_1 (and CB_2) receptors are activated by

the endogenous eicosanoids anandamide and 2-AG. Anandamide functions as a high affinity, moderate efficacy, selective agonist of CB_1, while 2-AG is a moderate affinity, high efficacy agonist of CB_1 and CB_2 (Di Marzo and De Petrocellis 2012). Unlike Δ^9-THC, which is a high affinity, moderate efficacy agonist of CB_1 and CB_2, other SCRAs show a range of affinities for both receptors but typically act as high efficacy agonists (Wiley et al. 2013; Gamage et al. 2018). Like Δ^9-THC, SCRA NPS are intended to elicit psychoactive effects by binding to the orthosteric site of CB_1, inducing receptor activation, and effecting signal transduction through several signaling pathways.

While activation of G proteins has been considered the most fundamental function of GPCRs, it is important to note that coupling to G proteins is only one of the ways in which GPCRs transduce signals across membranes, and direct interactions with other proteins such as arrestins and protein kinases are likely to be important mediators of drug actions at GPCRs, including $CB_1$and CB_2. CB_1 receptors couple readily to $G\alpha_i$ and $G\alpha_o$ heterotrimers, as well as to the related $G\alpha_z$. In circumstances where signaling to $G\alpha_{i/o}$ is blocked or saturated, activation of $G\alpha_s$ can be observed, and in some cell types, CB_1 can also activate $G\alpha_{q/11}$ (reviewed in Ibsen et al. 2017). By contrast, CB_2 predominantly couples through $G\alpha_{i/o}$, although there is evidence for activation of $G\alpha_{q/11}$. Activation of $G\alpha_{i/o/z}$ mediates inhibition of adenylyl cyclase (AC), while activation of $G\alpha_s$ stimulates AC, producing changes in the activity of effectors such as protein kinase A, cyclic adenosine monophosphate (cAMP)-gated ion channels, and exchange protein activated by cAMP (Epac). The primary consequence of activation of $G\alpha_{q/11}$ is stimulation of phospholipase C (PLC), which leads to mobilization of intracellular calcium stores and activation of protein kinase C – mediated, respectively, by the inositol trisphosphate and diacylglycerol liberated from the primary PLC substrate, phosphatidylinositol-4,5-bisphosphate. The βγ subunits of G protein heterotrimers directly activate inwardly rectifying potassium channels ($K_{IR}3.X$, GIRK) and inhibit voltage-gated calcium channels, as well as potentially contributing to regulation of isoforms of AC, PLC, and protein kinases.

Modulation of subsets of these signaling cascades is common to most GPCRs; the receptor-specific contributions are determined by the co-location of receptors and effectors, both with each other and within distinct cellular compartments and neuronal populations. For the most part, the predominant $G\alpha_{i/o}$ coupling of CB_1 and their location on nerve terminals means that the primary effect of activating the receptor is inhibition of neurotransmitter release. It is likely that one of the main functions of CB_1 in the CNS is the ad hoc regulation of synaptic transmission mediated by the activity-dependent generation of endocannabinoids. Inhibition of neurotransmitter release does not of course mean that neuronal activity is necessarily suppressed, as CB_1 is prominently located on both glutamatergic (excitatory) and GABAergic (inhibitory) neurons (Lovinger 2008).

The majority of studies investigating the signaling of CB_1 have been performed using Δ^9-THC, selected synthetic cannabinoids such as CP 55,940 or WIN 55,212-2, and the endogenous ligands 2-AG and AEA. Indeed, [^{3}H]CP 55,940 is one of the most commonly used radioligands for competition binding studies at CB_1 and serves as a point of reference for reported K_i and IC_{50} values. Functional activities (EC_{50}) for selected ligands have been reported using the [^{35}S]GTPγS assay and more recently with fluorescence-based membrane potential assays, with more limited investigation of functional selectivities. For example, HU-210 demonstrates maximal activation of G_i and G_o, while AEA and WIN 55,212-2 elicit full activation of G_i, and Δ^9-THC shows submaximal activation of both G_i and G_o (Glass and Northup 1999). WIN 55,212-2 signals through $G_{q/11}$ to increase intracellular calcium, whereas AEA, HU-210, and Δ^9-THC do not (Lauckner et al. 2005). In a mouse cell culture model in striatal medium spiny projection neurons, Δ^9-THC and CP 55,940 – but not AEA or WIN 55,212-2 – promoted CB_1 internalization via β-arrestin recruitment (Laprairie et al. 2014).

The key points from these studies are that Δ^9-THC has lower efficacy than the other compounds, and while there is some evidence for ligand-dependent preferential activation of signaling pathways, this is not an outstanding feature of cannabinoid ligands studied to date (Soethoudt et al. 2017). The implications of these findings are that while acute actions of cannabinoid ligands explored thus far are likely to be similar, in some situations where receptor reserve is low, Δ^9-THC may in fact act as a functional antagonist of endocannabinoid function rather than an endocannabinoid mimic. The differences in efficacy between Δ^9-THC and other ligands are likely to be most evident in situations of chronic cannabinoid use, where receptor desensitization and downregulation may occur; however, the molecular mechanisms underlying cellular adaptations to prolonged cannabinoid use remain obscure.

In humans and nonhuman animals, chronic administration of cannabinoid agonists leads to tolerance and dependence, and cessation produces a withdrawal syndrome characterized by anxiety and sleep disturbance (Lichtman and Martin 2005). Historically, frequent cannabis use was the only possible form of cannabinoid abuse and represents regular exposure to a single low efficacy CB_1 agonist (i.e., Δ^9-THC). The abuse of SCRA NPS, which comprise hundreds of typically high-efficacy CB_1 agonists of differing pharmacological profiles, is a recent phenomenon, and the long-term effects are unknown. The behavioral pharmacology of SCRAs in humans and nonhuman animals will be discussed in the next chapter.

4 Historical Synthetic Cannabinoid Receptor Agonists

Thousands of SCRAs with varying selectivity for CB_1 or CB_2 have been developed since the middle of the twentieth century by academic research laboratories and pharmaceutical companies (Thakur et al. 2009; Aghazadeh Tabrizi et al. 2016). A comprehensive structural survey of SCRAs is beyond the scope of this review, and only a subset have ever been identified as NPS. SCRAs can be classified into two

broad classes: those resembling phytocannabinoids and those derived from non-phytocannabinoid scaffolds. The phytocannabinoid analogs are sometimes subclassified as "classical" and "nonclassical" pending a tricyclic or bicyclic core, respectively, and were only encountered in the very earliest SCRA NPS products. Almost all SCRAs identified in NPS currently are non-phytocannabinoid derivatives.

4.1 Classical Cannabinoids and Phytocannabinoid Analogs

Despite an extensive history of human use, the structural elucidation of the psychoactive constituents of cannabis did not occur until the twentieth century (reviewed in Mechoulam and Hanus (2000)). Although researchers had isolated pharmacologically active extracts from cannabis since the mid-nineteenth century, the first pure phytocannabinoid, CBN, was isolated from a plant extract by a group at Cambridge in 1899 (Wood et al. 1899). CBN was erroneously believed to be the principal psychoactive component of cannabis at that time, despite its low abundance in cannabis, and was likely an oxidized degradant arising from isolation and storage.

Several groups were working contemporaneously to elucidate the components of cannabis by chemical derivatization and synthesis; however, conclusive confirmation of the structure of CBN by synthesis was first reported by Roger Adams and colleagues at the University of Illinois in 1940 (Adams et al. 1940a). Around the same time, the structure of CBD was resolved without stereochemical assignment and shown to readily isomerize to a pharmacologically active tetrahydrocannabinol (Adams et al. 1940b). Over the next decade, several tricyclic benzopyrans related to racemic $\Delta^{6a,10a}$-tetrahydrocannabinol ($\Delta^{6a,10a}$-THC, **15**, Fig. 2) were synthesized and demonstrated cannabimimetic activity. Homologation of $\Delta^{6a,10a}$-THC to hexyl (parahexyl, **16**) or heptyl (**17**) congeners increased activity, but further homologation or truncation decreased activity (Russell et al. 1941). Branching of the C-3 alkyl substituent enhanced activity, with many examples including the 1′,1′- (**18**) and 1′,2′-dimethylheptyl analogs (DMHP, **19**) showing greater potency in vivo than **17** itself, thereby providing the first SARs for synthetic phytocannabinoid analogs (Adams et al. 1948, 1949). DMHP was so potent, and had such a long duration of action, that it was explored as an incapacitating agent in humans by Edgewood Arsenal under the codename EA 1476 (National Research Council 1984; Huffman et al. 1997a).

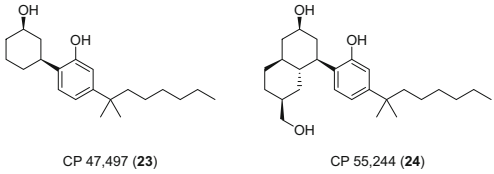
The conclusive stereochemical assignment of CBD and Δ^9-THC in 1963 and 1964, respectively, was aided by nuclear magnetic resonance spectroscopy techniques not available to earlier researchers (Mechoulam and Shvo 1963; Gaoni and Mechoulam 1964). This was followed by the total synthesis of the racemates of CBD and Δ^9-THC in 1965 (Mechoulam and Gaoni 1965). In 1972, 11-hydroxy-THC (**20**) was identified as the principal metabolite of Δ^9-THC and demonstrated comparable cannabimimetic potency following intravenous administration (Lemberger et al. 1972). Analgesic potency was retained by the simplified

11-OH-THC analog 9-nor-9β-hydroxyhexahydrocannabinol (HHC, **21**) (Bloom et al. 1977). It was possible to establish limited SARs for synthetic phytocannabinoid analogs in animals (Razdan 1986), however, the discovery of CB_1 in 1990 and the development of binding and functional assays facilitated the generation of SARs for hundreds of phytocannabinoid analogs.

Although Δ^9-THC is the more abundant in cannabis, the synthesis of phytocannabinoid analogs was often based on Δ^8-THC (**22**) since it is the more stable isomer and shows comparable cannabimimetic activity (Huffman et al. 1997b, 2003b). Building on earlier SAR, HU-210 (**9**) was first synthesized in 1988 and shown to be potently cannabimimetic in several species, with 100–800 times greater activity in mice than Δ^9-THC (Mechoulam et al. 1988; Little et al. 1989; Järbe et al. 1989). Despite decades of drug development focused on synthetic phytocannabinoid analogs and the synthesis of hundreds of classical cannabinoids, only two SCRAs are currently marketed therapeutic agents. The synthetic form of Δ^9-THC (dronabinol, Marinol®) was developed by Unimed Pharmaceuticals (Solvay) and approved by the US Food and Drug Administration as an antiemetic for chemotherapy-induced nausea and vomiting, and the SCRA nabilone (**8**, Cesamet®) was developed by Eli Lily and Company for the same indication. Currently, none of the classical cannabinoids have been identified as SCRA NPS, likely due to the nontrivial synthesis of such compounds.

4.2 Nonclassical Cannabinoids

Following the development of classical cannabinoid SARs in the preceding decades, the major pharmaceutical firms had established cannabinoid development programs by the 1970s. In the 1970s and 1980s, Pfizer undertook systematic deconstruction and elaboration of Δ^9-THC, filing patents around libraries of compounds lacking the pyran ring of classical cannabinoids (Harbert et al. 1981; Johnson and Melvin 1983). A simplified bicyclic analog of HHC and HU-210, (±)-*cis*-CP 47,497 (**23**, Fig. 3), demonstrated analgesic effects in vivo and substituted for Δ^9-THC (Weissman et al. 1982). Consistent with trends in the corresponding classical cannabinoids, the C8 homolog of CP 47,497 (CP 47,497-C8, cannabicyclohexanol, CCH, **11**) was the most potent in the homologous series (Compton et al. 1992b, 1993). Related nonclassical cannabinoids, such as CP 55,940 (**12**) and CP 55,244 (**24**), also showed analgesic activity against chemical, electrical, and thermal nociceptive stimuli in mice and rats.



CP 47,497 (**23**) CP 55,244 (**24**)

Fig. 3 Nonclassical synthetic cannabinoid receptor agonists

While CP 55,940 remains one of the most widely used tool molecules in cannabinoid pharmacology research, **11** appeared from obscurity as one of the first SCRA NPS. Like the classical cannabinoids, the nonclassical cannabinoids are synthetically demanding and would ultimately be replaced on the SCRA NPS market by simpler CB_1 agonists.

4.3 Aminoalkylindoles (AAIs)

In the late 1980s, a number of aminoalkylindoles (AAIs) with analgesic and anti-inflammatory properties were disclosed by pharmaceutical firm Sterling Winthrop, including pravadoline (WIN 48,098, **25**, Fig. 4), its naphthyl analog (**26**), and WIN 55,225 (**27**) (Bell 1986). Pravadoline was developed as a nonsteroidal anti-inflammatory drug (NSAID), and its ability to inhibit prostaglandin synthesis was rationalized by its structural similarity to known cyclooxygenase inhibitors such as ketoprofen, indomethacin, and clometacin. Pravadoline provided analgesia against a more diverse range of nociceptive stimuli than traditional NSAIDs, at concentrations below the effective anti-inflammatory dose, and these effects were not reversed by an opioid antagonist (Haubrich et al. 1990). Furthermore, naphthyl analog **26** and WIN 55,225 retained analgesic activity without COX inhibition, suggesting a unique mechanism of action (Bell et al. 1991). The antinociceptive potency of AAIs was known to correlate with their ability to inhibit smooth muscle contractions in vitro, a capability shared by many AAIs but not structurally disparate COX inhibitors (Ward et al. 1990). AAIs were found to inhibit adenylyl cyclase in specific regions of rat brain through a G protein mechanism with the same rank-order potency as their inhibitory action on tissue preparations (Pacheco et al. 1991). These activities of AAIs were stereospecific, as exemplified by the efficacy of WIN 55,212-2 (**13**) but not its enantiomer, suggesting that AAIs interact with a specific GPCR in the brain and periphery. Moreover, several AAIs shared similar pharmacological profiles with Δ^9-THC in vivo (Compton et al. 1992a).

Following the identification of the CB_1 receptor, the SAR of pravadoline with respect to substituents at the 1-, 2-, and 3-positions of the indole ring was extensively explored in competition binding assays against [^{3}H]WIN 55,212-2 (D'Ambra et al. 1991). Incorporation of the 1-naphthyl group of WIN 55,212-2 gave **26**

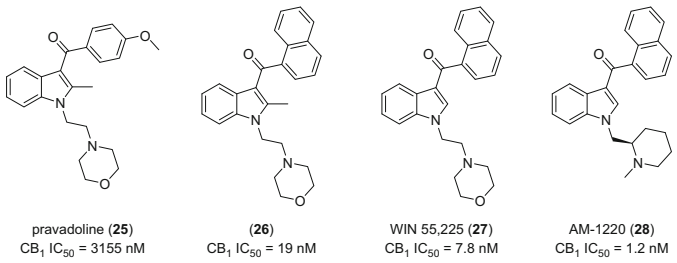


Fig. 4 Aminoalkylindoles (AAIs) developed by Sterling Winthrop

(IC_{50} = 19 nM), with improved CB_1 affinity over pravadoline (IC_{50} = 3,155 nM), and removal of the C-2 methyl group further improved affinity (**27**, IC_{50} = 7.8 nM) (Eissenstat et al. 1995). Exploration of regioisomerism and simplication of the morpholine unit produced the compound later named AM-1220 (**28**), with activity residing solely in the depicted (*R*)-enantiomer (D'Ambra et al. 1996).

4.3.1 Naphthoylindoles

In order to develop SARs for the recently identified CB_1 receptor, the laboratory of John Huffman at Clemson University synthesized hundreds of indole, indazole, and pyrrole derivatives to systematically identify structural requirements for binding at the CB_1 orthosteric site (Huffman and Padgett 2005). The importance of alkyl chain length at *N*-1 was demonstrated by a series of homologs featuring methyl through heptyl substituents (Fig. 5) (Huffman et al. 1994; Wiley et al. 1998; Aung et al. 2000). Although methyl, ethyl, and propyl groups conferred only micromolar CB_1 affinity, butyl (JWH-073, **29**, K_i = 8.90 nM), pentyl (JWH-018, **14**, K_i = 9.00 nM), and hexyl (JWH-019, **30**, K_i = 9.80 nM) groups resulted in nanomolar CB_1 affinity. Further homologation of JWH-019 to the *N*-heptyl derivative (JWH-020, **31**, K_i = 128 nM) decreased CB_1 affinity. In all cases, selectivity for CB_1 over CB_2 was low.

Alexandros Makriyannis and colleagues at the University of Connecticut were also exploring naphthoylindole cannabinoids and reported in a patent that terminal fluorination of the pentyl chain of JWH-018 improved CB_1 affinity (AM-2201, **32**, K_i = 1.0 nM), as did installation of a terminal nitrile group (AM-2232, **33**, K_i = 0.28 nM) (Makriyannis and Deng 2001).

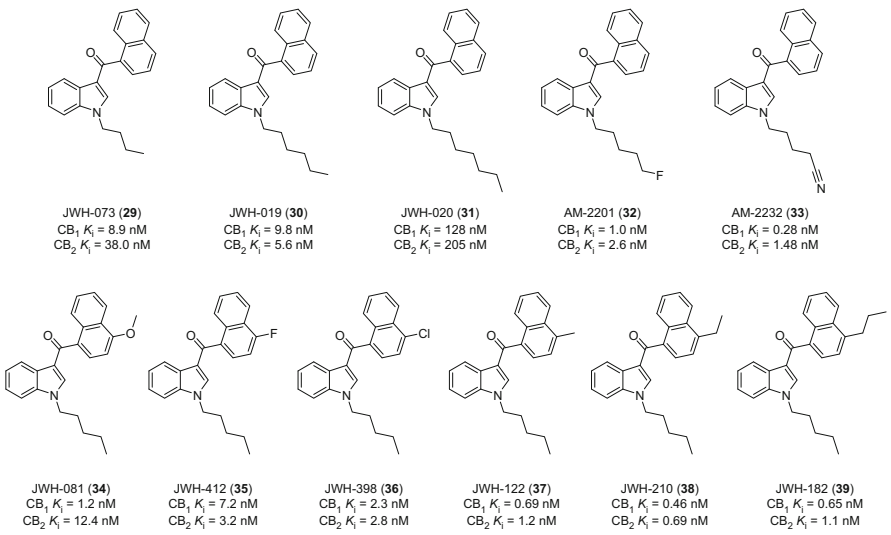


Fig. 5 Selected naphthoylindole SCRAs

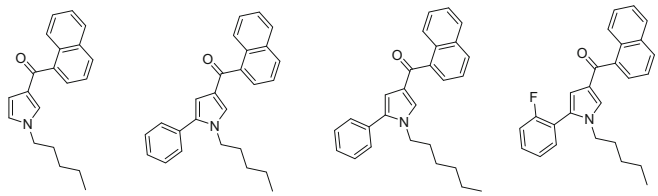


Fig. 6 Selected naphthoylpyrrole SCRAs

The effect of naphthalene substituents on CB_1 and CB_2 binding was explored, and electron-donating and electron-withdrawing groups (EDGs and EWGs) were tolerated (Aung et al. 2000; Huffman et al. 2003a, 2005b; Smith 2008; Wiley et al. 2012). In the 4-position, a methoxy group (JWH-081, **34**, $K_i = 1.2$ nM) conferred better CB_1 affinity than a fluoro- (JWH-412, **35**, $K_i = 7.2$ nM) or chloro-substituent (JWH-398, **36**, $K_i = 2.3$ nM), indicating a potential steric requirement for optimal CB_1 binding. Indeed, CB_1 binding tolerated a methyl (JWH-122, **37**, $K_i = 0.69$ nM), ethyl (JWH-210, **38**, $K_i = 0.46$ nM), and propyl (JWH-182, **39**, $K_i = 0.65$ nM) moiety equally well.

4.3.2 Naphthoylpyrroles

The indole group of JWH-018 is not strictly required for CB_1 binding, and series of homologous pyrrole analogs demonstrated reduced CB_1 affinities compared to the corresponding indoles (Fig. 6). The highest affinity was observed for *N*-pentyl derivative JWH-030 (**40**, $K_i = 87$ nM), an order of magnitude lower than JWH-018, with truncation or homologation reducing CB_1 binding (Lainton et al. 1995; Wiley et al. 1998). JWH-030 showed reduced maximal efficacy in the [^{35}S]GTPγS assay when compared to many other SCRAs, including naphthoylindoles, indicating that it functioned as a low efficacy agonist like Δ^9-THC (Griffin et al. 1998). Installation of a 2-phenyl group partially restored CB_1 affinity, as in JWH-145 (**41**, $K_i = 14$ nM), and *N*-hexyl derivative JWH-147 (**42**, $K_i = 11$ nM) had a comparable profile (Huffman et al. 2006). Further exploration of aryl substituents in JWH-145 showed that small electronegative groups in the *ortho*-position enhanced CB_1 affinity, as observed for JWH-307 (**43**, $K_i = 7.7$ nM), but larger aliphatic groups and regioisomers were not tolerated.

4.3.3 Phenylacetylindoles and Benzoylindoles

A series of phenylacetylindoles and benzoylindoles approximating removal of the respective proximal or distal six-membered ring of naphthoylindoles was developed to probe aromatic stacking in a model of CB_1 binding (Figs. 7 and 8). The simple phenylacetylindole JWH-167 (**44**, $K_i = 90$ nM) demonstrated CB_1 binding that was an order of magnitude lower than JWH-018 (Huffman et al. 2005a). CB_1 binding was improved by various substituents in the 2- and 3-positions of the phenyl ring,

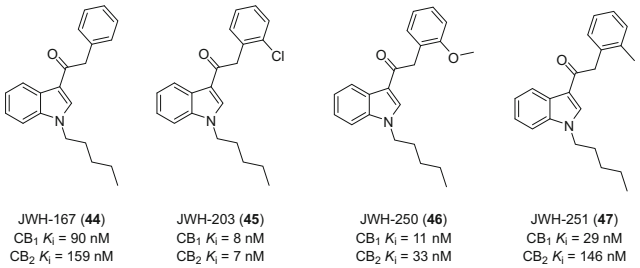


Fig. 7 Selected phenylacetylindole SCRAs

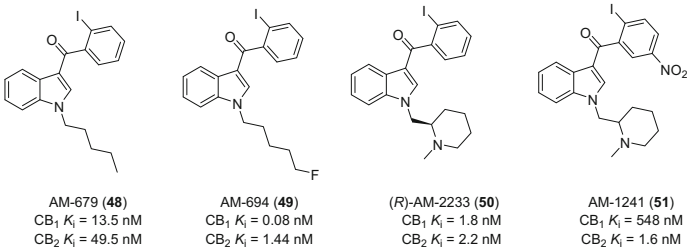


Fig. 8 Selected benzoylindole SCRAs

such as JWH-203 (**45**, $K_i = 8$ nM), JWH-250 (**46**, $K_i = 11$ nM), and JWH-251 (**47**, $K_i = 29$ nM), but not the 4-position.

Iodinated benzoylindoles were initially developed by Makriyannis and others to explore CB_1 SAR as well as to facilitate CB_1 imaging via iodine-131-labeled isotopologues (Deng et al. 2005). One of the earliest examples disclosed was AM-679 (**48**, $K_i = 13.5$ nM), containing the *N*-pentyl chain of JWH-018, terminal fluorination of which produced AM-694 (**49**, $K_i = 0.08$ nM) that produced a profound increase in binding affinity (Makriyannis and Deng 2001). Other *N*-1 substituents were also explored, including the *N*-methylpiperidine group of earlier AAIs. *(R)*-AM-2233 (**50**, $K_i = 1.8$ nM) demonstrated nanomolar CB_1 affinity, while AM-1241 (**51**, $K_i = 548$ nM) was a relatively selective CB_2 ligand (Deng 2000).

4.3.4 Alicyclic and Polycyclic 3-Acylindoles

Aromaticity of the pendant ketone substituent is not a strict requirement for CB_1 binding, and SCRAs featuring bulky alicyclic (e.g., 2,2,3,3-tetramethylcyclopropane) or polycyclic (e.g., adamantane) groups have been described (Fig. 9). AM-1248 (**52**) was first reported as a moderate affinity CB_1 ($K_i = 100$ nM) ligand by Makriyannis in a 2001 patent (Makriyannis and Deng 2001), and a 2006 patent granted to Abbott Laboratories details numerous 3-acylindoles featuring a 2,2,3,3-tetramethylcyclopropyl (TMCP) unit as CB_2 ligands for the treatment of pain, including *N*-methylpiperidine analog AB-005 (**53**, $K_i = 5.5$ nM) (Pace et al. 2006). Further exploration of SARs for this class of

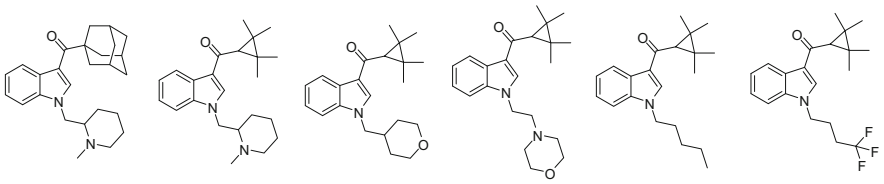


Fig. 9 Selected alicyclic and polycyclic acylindole SCRAs

SCRAs showed that many substituents were tolerated at the 1-position without diminishing CB_2 selectivity; however, many retained sufficient CB_1 affinity to be considered SCRAs (Frost et al. 2008, 2010). Replacing the *N*-methylpiperidine unit of AB-005 with a tetrahydropyran (THP) was tolerated at CB_1 (A-834,735, **54**, $K_i = 12$ nM), but more extended substituents at the 1-position were detrimental, as evidenced by A-796,260 (**55**, $K_i = 845$ nM) and UR-144 (**56**, $K_i = 150$ nM). This departure from the optimal C4–C6 chain established for the JWH series suggests an alternative binding mode for TMCP-3-acylindole SCRAs; however, CB1 affinity was retained in a terminally trifluorinated *N*-butyl analog (XLR-12, **57**, $K_i = 15$ nM).

5 Synthetic Cannabinoid Receptor Agonists as New Psychoactive Substances

Around 2004, the herbal product known as "Spice" became widely available in Europe as a so-called legal high with cannabis-like properties when smoked. By 2008, a German forensic laboratory had determined that the psychoactive constituents of this product were CP 47,497-C8 and JWH-018 (Auwärter et al. 2009). As governments enacted legislation prohibiting or restricting these two substances, they were soon replaced with other SCRAs previously reported in the scientific literature (King 2014). This "cat-and-mouse" cycle of reactive SCRA prohibition and subsequent release of unregulated analogs continued for several years starting in the late 2000s, and many of the JWH and AM series SCRAs have been encountered as NPS, including all of the SCRAs depicted in Figs. 5, 6, 7, 8, and 9. Estimates of SCRA prevalence have been reported for several countries based on trends in law enforcement seizures, forensic analyses, and clinical toxicology (Chung et al. 2014; Hermanns-Clausen et al. 2013; Winstock and Barratt 2013; Zuba and Byrska 2013; Kikura-Hanajiri et al. 2014; Langer et al. 2014, 2016a, b).

Analysis of SCRA products conducted in Japan between 2009 and 2013 showed that CCH, CP-47,497, and JWH-018 were most frequently detected until they were prohibited in November 2009 and replaced by JWH-073 and JWH-250 (Kikura-Hanajiri et al. 2013). Following prohibition of JWH-073 and JWH-250 in September 2010, other SCRAs with higher CB_1 binding affinities, such as JWH-081, JWH-122, JWH-210, and AM-694, were encountered. Generic scheduling of

naphthoylindole SCRAs and analogs in November 2012 produced a gradual disappearance of JWH-type compounds from the NPS market until March 2013 when they had been entirely replaced by new SCRA chemotypes (Kikura-Hanajiri et al. 2014).

Emergency scheduling of selected SCRAs in the USA by the Drug Enforcement Administration (DEA) had similar effects on the SCRA NPS market. According to the National Forensic Laboratory Information System (NFLIS), during the first half of 2010 most SCRAs were JWH compounds, predominantly JWH-018 (86%) (US Drug Enforcement Administration 2014). By 2012, JWH compounds accounted for less than 25% of SCRAs, and that dropped to less than 10% in 2013, with a concomitant rise in the detection of replacement SCRAs such as UR-144 and its fluorinated analog, XLR-11 (see Fig. 11). During the first half of 2012 almost 50% of all SCRA seizures were AM-2201, and by the first half of 2013 about 65% were XLR-11. XLR-11 was the second most frequently encountered SCRA after AM-2201 in Arkansas from January 2010 through December 2012 (Seely et al. 2013). In Indiana, AM-2201 was found in 70% of products prior to its designation as a substance listed in Schedule I of the US Controlled Substances Act in 2012, after which SCRA products were found to contain predominantly UR-144 (65% of products), XLR-11 (42%), and A-796,260 (19%) (Shanks et al. 2013).

Similarly, a change of German regulations in July 2012 restricting the availability of many of the earliest SCRAs resulted in an increased prevalence of TMCP SCRAs, including UR-144 and XLR-11 (Langer et al. 2014). In Korea in 2013, following legislative changes targeting JWH- and AM-type SCRAs, XLR-11 became the most frequently encountered SCRA despite appearing in Korea for the first time in 2012 (Chung et al. 2014). Similar trends have been observed elsewhere (Langer et al. 2014, 2016a, b; Zuba and Byrska 2013). By 2012, several putative SCRAs with no precedent in the scientific literature were identified as NPS. These SCRAs appeared to be the products of rational design; they were produced using traditional medicinal chemistry techniques such as molecular hybridization, bioisosteric replacement, and scaffold hopping.

5.1 Molecular Hybridization

Molecular hybridization is a technique commonly used in medicinal chemistry to generate a new chemical entity by recombination of structural or pharmacophoric subunits from two or more molecules (Viegas-Junior et al. 2007). The earliest identified SCRA NPS without precedent in the scientific literature at the time of their discovery were typically combinations of functional groups previously reported for known CB_1 ligands. For example, cannabipiperidiethanone (CPE, **58**, IC_{50} = 591 nM, Fig. 10) is a molecular hybrid of JWH-250 and AM-1220 and was found alongside JWH-122 and JWH-081 (Uchiyama et al. 2011, 2012).

RCS-4 (**59**), a hybrid of JWH-081 and AM-679, was identified as a CB_1 agonist (EC_{50} = 146 nM) along with regioisomeric RCS-2 (**60**, EC_{50} = 54 nM) and homolog RCS-4-C4 (**61**, EC_{50} = 574 nM) following their detection in Belgium,

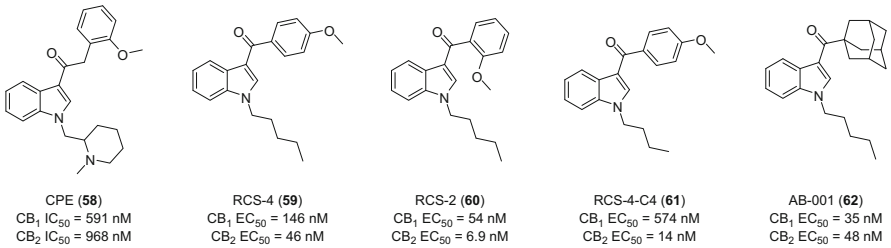


Fig. 10 Selected SCRA molecular hybrids

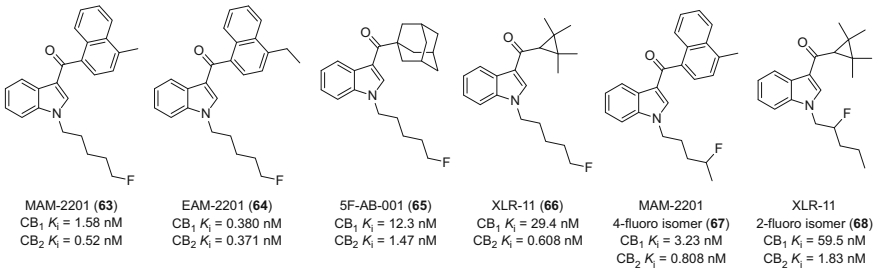


Fig. 11 Selected fluorinated SCRAs

Japan, Korea, and New Zealand between 2011 and 2013 (Nakajima et al. 2011a, b; Couch and Madhavaram 2012; Denooz et al. 2013; Park et al. 2013; Banister et al. 2015a). AB-001 (**62**) is a molecular hybrid of JWH-018 and AM-1248 and was unknown prior to its discovery in Hungary in 2011 (Jankovics et al. 2012). Although the cannabimimetic activity of AB-001 was confirmed in vitro ($EC_{50} = 35$ nM), its potency in rats was much lower than Δ^9-THC or JWH-018 and showed only threshold bradycardic and hypothermic trends at 30 mg/kg (Banister et al. 2013).

5.2 Bioisosteric Fluorination

Fluorine is one of the most widely employed bioisosteric replacements for hydrogen in pharmaceuticals and frequently confers improved potency and/or metabolic stability (Gillis et al. 2015). The use of fluorine-for-hydrogen bioisosteric replacement has been explored in most other classes of NPS, such as hallucinogenic phenethylamines and amphetamine/cathinone stimulants (Trachsel 2012). The terminal fluorination of *N*-pentyl-3-acylindole SCRAs is a trend that likely originated due to the greater CB_1 binding affinity of AM-2201 compared to JWH-018, and several groups have systematically explored the effects of this bioisosteric replacement on cannabimimetic activity in SCRA NPS (Wilkinson et al. 2015; Banister et al. 2015b; Hess et al. 2016). This structural modification has persisted and will be explored in more detail in the next chapter, but some early examples (circa 2012)

include terminal fluorination of JWH-122 and JWH-210 to give the unprecedented analogs MAM-2201 (Fig. 11, **63**, $K_i = 1.58$ nM) and EAM-2201 (**64**, $K_i = 0.38$ nM), respectively, with little change to CB_1 affinity in either case (Simolka et al. 2012; Uchiyama et al. 2013a; Hess et al. 2016). The corresponding fluorinated analogs of AB-001 and UR-144, 5F-AB-001 (**65**, $K_i = 12.3$ nM), and XLR-11 (**66**, $K_i = 29.4$ nM), respectively, were unknown at the time of detection (Uchiyama et al. 2013b). AB-001 and 5F-AB-001 had a short lifetime on the NPS market, and based on the low potency of AB-001 in rats, it is possible that both had physicochemical profiles that limited brain penetration and psychoactive effects. In contrast, XLR-11 remained one of the most prevalent SCRAs in many parts of the world (even after being placed under legislative control) although more potent and unregulated SCRAs had since emerged.

Other examples of bioisosteric replacement include nonterminal fluorination of various *N*-pentyl SCRAs, such as the 4-fluoro isomer of MAM-2201 (**67**, $K_i = 3.23$ nM) and the 2-fluoro isomer of XLR-11 (**68**, $K_i = 59.5$ nM) (Hess et al. 2016). Such positional isomers are likely intended to obfuscate analytical analysis while retaining cannabimimetic potency.

6 Conclusions

As reactive legal provisions attempted to control specific SCRA NPS in many jurisdictions starting around 2010, manufacturers responded by releasing increasingly diverse substitutes. Structural features that had not previously been reported for SCRAs appeared in some examples, such as the cyclohexylethyl group of RCS-8 (**69**, $K_i = 81.3$ nM, Fig. 12) and the 3-pyridinyl unit of (**70**) (no pharmacological data reported) (Logan et al. 2012; Uchiyama et al. 2013a; Hess et al. 2016). SCRA NPS designers have also moved beyond the 3-acylindole core of most compounds in this chapter to incorporate ester and amide linkers, new heteroaromatic scaffolds, and diversified pendant groups (e.g., BB-22, **71**; AB-FUBINACA, **72**).

The ad hoc design of new SCRA NPS by mining the scientific and patent literature for new cannabinoid ligands and conducting essentially random recombination of subunits has exponentially increased the number of SCRA NPS identified in the marketplace. Unlike earlier SCRA NPS with precedent in the scientific

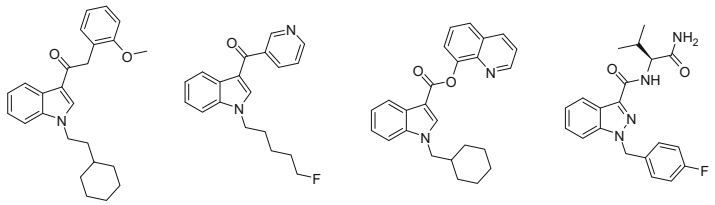


Fig. 12 Selected SCRAs featuring novel structural subunits

literature, newer SCRA NPS are often completely novel, and no preclinical pharmacological data are available in many cases. In some cases, metabolically reactive functional groups or other toxicophoric elements are present. It is perhaps unsurprising that the evolution of SCRA NPS has trended toward increasingly potent compounds and more frequent mass casualty events. The following chapter will explore the chemistry and pharmacology of this evolving class of NPS and the potential consequences for human health due to abuse of these substances.

References

Adams R, Baker BR, Wearn RB (1940a) Structure of cannabinol. III. Synthesis of cannabinol, 1-hydroxy-3-n-amyl-6,6,9-trimethyl-6-dibenzopyran1. J Am Chem Soc 62:2204–2207

Adams R, Loewe S, Pease DC, Cain CK, Wearn RB, Baker RB, Wolff H (1940b) Structure of cannabidiol. VIII. Position of the double bonds in cannabidiol. Marihuana activity of tetrahydrocannabinols. J Am Chem Soc 62:2566–2567

Adams R, Mackenzie S, Loewe S (1948) Tetrahydrocannabinol homologs with doubly branched alkyl groups in the 3-position. XVIII1. J Am Chem Soc 70:664–668

Adams R, Harfenis M, Loewe S (1949) New analogs of tetrahydrocannabinol. XIX. J Am Chem Soc 71:1624–1628

Aghazadeh Tabrizi M, Baraldi PG, Borea PA, Varani K (2016) Medicinal chemistry, pharmacology, and potential therapeutic benefits of cannabinoid CB2 receptor agonists. Chem Rev 116:519–560

Angerer V, Moosmann B, Franz F, Auwärter V (2015) 5F-cumyl-PINACA in 'e-liquids' for electronic cigarettes – a new type of synthetic cannabinoid in a trendy product. In: 53rd Annual meeting of the International Association of Forensic Toxicologists (TIAFT), Firenze, Italy

Aung MM, Griffin G, Huffman JW, Wu MJ, Keel C, Yang B, Showalter VM, Abood ME, Martin BR (2000) Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB1 and CB2 receptor binding. Drug Alcohol Depend 60:133–140

Auwärter V, Dresen S, Weinmann W, Muller M, Putz M, Ferreiros N (2009) 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? J Mass Spectrom 44:832–837

Banister SD, Wilkinson SM, Longworth M, Stuart J, Apetz N, English K, Brooker L, Goebel C, Hibbs DE, Glass M, Connor M, Mcgregor IS, Kassiou M (2013) The synthesis and pharmacological evaluation of adamantane-derived indoles: cannabimimetic drugs of abuse. ACS Chem Neurosci 4:1081–1092

Banister SD, Stuart J, Conroy T, Longworth M, Manohar M, Beinat C, Wilkinson SM, Kevin RC, Hibbs DE, Glass M, Connor M, Mcgregor IS, Kassiou M (2015a) Structure–activity relationships of synthetic cannabinoid designer drug RCS-4 and its regioisomers and C4 homologues. Forensic Toxicol 33:355–366

Banister SD, Stuart J, Kevin RC, Edington A, Longworth M, Wilkinson SM, Beinat C, Buchanan AS, Hibbs DE, Glass M, Connor M, Mcgregor IS, Kassiou M (2015b) Effects of bioisosteric fluorine in synthetic cannabinoid designer drugs JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135. ACS Chem Neurosci 6:1445–1458

Bell, MR (1986) 3-Arylcarbonyl- and 3-cycloalkylcarbonyl-1-aminoalkyl-1H-indoles, compositions and use. US Patent 4,581,354

Bell MR, D'ambra TE, Kumar V, Eissenstat MA, Herrmann JL Jr, Wetzel JR, Rosi D, Philion RE, Daum SJ, Hlasta DJ, Kullnig RK, Ackerman JH, Haubrich DR, Luttinger DA, Baizman ER, Miller MS, Ward SJ (1991) Antinociceptive (aminoalkyl)indoles. J Med Chem 34:1099–1110

Bloom AS, Dewey WL, Harris LS, Brosius KK (1977) 9-Nor-9beta-hydroxyhexahydrocannabinol, a cannabinoid with potent antinociceptive activity: comparisons with morphine. J Pharmacol Exp Ther 200:263–270

Busquets GA, Soria-Gomez E, Bellocchio L, Marsicano G (2016) Cannabinoid receptor type-1: breaking the dogmas [version 1; referees: 3 approved]. F1000Research 5:990

Chung H, Choi H, Heo S, Kim E, Lee J (2014) Synthetic cannabinoids abused in South Korea: drug identifications by the National Forensic Service from 2009 to June 2013. Forensic Toxicol 32:82–88

Compton DR, Gold LH, Ward SJ, Balster RL, Martin BR (1992a) Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from delta 9-tetrahydrocannabinol. J Pharmacol Exp Ther 263:1118–1126

Compton DR, Johnson MR, Melvin LS, Martin BR (1992b) Pharmacological profile of a series of bicyclic cannabinoid analogs: classification as cannabimimetic agents. J Pharmacol Exp Ther 260:201–209

Compton DR, Rice KC, De Costa BR, Razdan RK, Melvin LS, Johnson MR, Martin BR (1993) Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities. J Pharmacol Exp Ther 265: 218–226

Couch RF, Madhavaram H (2012) Phenazepam and cannabinomimetics sold as herbal highs in New Zealand. Drug Test Anal 4:409–414

D'ambra TE, Bacon ER, Bell MR, Carabateas PM, Eissenstat MA, Kumar V, Mallamo JP, Ward SJ (1991) 3-Arylcarbonyl-1-(c-attached-n-heteryl)-1h-indoles. US Patent 5,068,234

D'ambra TE, Eissenstat MA, Abt J, Ackerman JH, Bacon ER, Bell MR, Carabateas PM, Josef KA, Kumar V, Weaver JD, Arnold R, Casiano FM, Chippari SM, Haycock DA, Kuster JE, Luttinger DA, Stevenson JI, Ward SJ, Hill WA, Khanolkar A, Makriyannis A (1996) C-attached aminoalkylindoles: potent cannabinoid mimetics. Bioorg Med Chem Lett 6:17–22

Deng H (2000) Design and synthesis of selective cannabinoid receptor ligands: aminoalkylindole and other heterocyclic analogs. PhD dissertation, University of Connecticut

Deng H, Gifford AN, Zvonok AM, Cui G, Li X, Fan P, Deschamps JR, Flippen-Anderson JL, Gatley SJ, Makriyannis A (2005) Potent cannabinergic indole analogues as radioiodinatable brain imaging agents for the CB1 cannabinoid receptor. J Med Chem 48:6386–6392

Denooz R, Vanheugen JC, Frederich M, De Tullio P, Charlier C (2013) Identification and structural elucidation of four cannabimimetic compounds (RCS-4, AM-2201, JWH-203 and JWH-210) in seized products. J Anal Toxicol 37:56–63

Devane W, Hanus L, Breuer A, Pertwee R, Stevenson L, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949

Di Marzo V (2008) Endocannabinoids: synthesis and degradation. Rev Physiol Biochem Pharmacol 160:1–24

Di Marzo V, De Petrocellis L (2012) Why do cannabinoid receptors have more than one endogenous ligand? Philos Trans R Soc Lond Ser B Biol Sci 367:3216–3228

Dresen S, Ferreiros N, Putz M, Westphal F, Zimmermann R, Auwarter V (2010) Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. J Mass Spectrom 45:1186–1194

Eissenstat MA, Bell MR, D'ambra TE, Alexander EJ, Daum SJ, Ackerman JH, Gruett MD, Kumar V, Estep KG (1995) Aminoalkylindoles: structure-activity relationships of novel cannabinoid mimetics. J Med Chem 38:3094–3105

Elsohly MA, Radwan MM, Gul W, Chandra S, Galal A (2017) Phytochemistry of Cannabis sativa L. In: King AD, Falk H, Gibbons S, Kobayashi JI (eds) Phytocannabinoids: unraveling the complex chemistry and pharmacology of Cannabis sativa. Springer, Cham

EMCDDA (2017) Synthetic cannabinoids in Europe. Perspectives on Drugs. Lisbon, Portugal. https://doi.org/10.2810/32306. http://www.emcdda.europa.eu/topics/pods/synthetic-cannabinoids_en. Accessed 19 Nov 2017

Frost JM, Dart MJ, Tietje KR, Garrison TR, Grayson GK, Daza AV, El-Kouhen OF, Miller LN, Li L, Yao BB, Hsieh GC, Pai M, Zhu CZ, Chandran P, Meyer MD (2008) Indol-3-yl-tetramethylcyclopropyl ketones: effects of indole ring substitution on CB2 cannabinoid receptor activity. J Med Chem 51:1904–1912

Frost JM, Dart MJ, Tietje KR, Garrison TR, Grayson GK, Daza AV, El-Kouhen OF, Yao BB, Hsieh GC, Pai M, Zhu CZ, Chandran P, Meyer MD (2010) Indol-3-ylcycloalkyl ketones: effects of N1 substituted indole side chain variations on CB(2) cannabinoid receptor activity. J Med Chem 53:295–315

Galiègue S, Mary S, Marchand J, Dussossoy D, Carrière D, Carayon P, Bouaboula M, Shire D, Le Fur G, Casellas P (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 232:54–61

Gamage TF, Farquhar CE, Lefever TW, Marusich JA, Kevin RC, McGregor IS, Wiley JL, Thomas BF (2018) Molecular and behavioral pharmacological characterization of abused synthetic cannabinoids MMB- and MDMB-FUBINACA, MN-18, NNEI, CUMYL-PICA, and 5-Fluoro-CUMYL-PICA. J Pharmacol Exp Ther 365:437–446

Gaoni Y, Mechoulam R (1964) Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc 86:1646–1647

Gérard CM, Mollereau C, Vassart G, Parmentier M (1991) Molecular cloning of a human cannabinoid receptor which is also expressed in testis. Biochem J 279:129–134

Gertsch J, Pertwee RG, Di Marzo V (2010) Phytocannabinoids beyond the cannabis plant – do they exist? Br J Pharmacol 160:523–529

Gillis EP, Eastman KJ, Hill MD, Donnelly DJ, Meanwell NA (2015) Applications of fluorine in medicinal chemistry. J Med Chem 58:8315–8359

Glass M, Northup JK (1999) Agonist selective regulation of G proteins by cannabinoid CB_1 and CB_2 receptors. Mol Pharmacol 56:1362–1369

Griffin G, Atkinson PJ, Showalter VM, Martin BR, Abood ME (1998) Evaluation of cannabinoid receptor agonists and antagonists using the guanosine-5′-O-(3-[^{35}S]thio)-triphosphate binding assay in rat cerebellar membranes. J Pharmacol Exp Ther 285:553–560

Harbert CA, Johnson MR, Melvin LS (1981) 3-[2-Hydroxy-4-(substituted)phenyl]-cycloalkanol analgesic agents. US Patent 4,306,097

Haubrich DR, Ward SJ, Baizman E, Bell MR, Bradford J, Ferrari R, Miller M, Perrone M, Pierson AK, Saelens JK (1990) Pharmacology of pravadoline: a new analgesic agent. J Pharmacol Exp Ther 255:511–522

Herkenham M, Lynn A, Johnson M, Melvin L, De Costa B, Rice K (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11:563–583

Hermanns-Clausen M, Kneisel S, Szabo B, Auwärter V (2013) Acute toxicity due to the confirmed consumption of synthetic cannabinoids: clinical and laboratory findings. Addiction 108:534–544

Hess C, Schoeder CT, Pillaiyar T, Madea B, Muller CE (2016) Pharmacological evaluation of synthetic cannabinoids identified as constituents of spice. Forensic Toxicol 34:329–343

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54:161–202

Hua T, Vemuri K, Pu M, Qu L, Han GW, Wu Y, Zhao S, Shui W, Li S, Korde A, Laprairie RB, Stahl EL, Ho JH, Zvonok N, Zhou H, Kufareva I, Wu B, Zhao Q, Hanson MA, Bohn LM, Makriyannis A, Stevens RC, Liu ZJ (2016) Crystal structure of the human cannabinoid receptor CB1. Cell 167:750–762

Hua T, Vemuri K, Nikas SP, Laprairie RB, Wu Y, Qu L, Pu M, Korde A, Jiang S, Ho JH, Han GW, Ding K, Li X, Liu H, Hanson MA, Zhao S, Bohn LM, Makriyannis A, Stevens RC, Liu ZJ (2017) Crystal structures of agonist-bound human cannabinoid receptor CB1. Nature 547:468–471

Huffman JW, Padgett LW (2005) Recent developments in the medicinal chemistry of cannabimimetic indoles, pyrroles and indenes. Curr Med Chem 12:1395–1411

Huffman JW, Dai D, Martin BR, Compton DR (1994) Design, synthesis and pharmacology of cannabimimetic indoles. Bioorg Med Chem Lett 4:563–566

Huffman JW, Duncan SG, Wiley JL, Martin BR (1997a) Synthesis and pharmacology of the 1′,2′-dimethylheptyl-Δ^8-THC isomers: exceptionally potent cannabinoids. Bioorg Med Chem Lett 7:2799–2804

Huffman JW, Lainton JH, Kenneth Banner W, Duncan SG, Jordan RD, Yu S, Dai D, Martin BR, Wiley JL, Compton DR (1997b) Side chain methyl analogues of Δ^8-THC. Tetrahedron 53:1557–1576

Huffman JW, Mabon R, Wu M-J, Lu J, Hart R, Hurst DP, Reggio PH, Wiley JL, Martin BR (2003a) 3-Indolyl-1-naphthylmethanes: new cannabimimetic indoles provide evidence for aromatic stacking interactions with the CB1 cannabinoid receptor. Bioorg Med Chem 11:539–549

Huffman JW, Miller JRA, Liddle J, Yu S, Thomas BF, Wiley JL, Martin BR (2003b) Structure–activity relationships for 1′,1′-dimethylalkyl-Δ8-tetrahydrocannabinols. Bioorg Med Chem 11:1397–1410

Huffman JW, Szklennik PV, Almond A, Bushell K, Selley DE, He H, Cassidy MP, Wiley JL, Martin BR (2005a) 1-Pentyl-3-phenylacetylindoles, a new class of cannabimimetic indoles. Bioorg Med Chem Lett 15:4110–4113

Huffman JW, Zengin G, Wu M-J, Lu J, Hynd G, Bushell K, Thompson ALS, Bushell S, Tartal C, Hurst DP, Reggio PH, Selley DE, Cassidy MP, Wiley JL, Martin BR (2005b) Structure–activity relationships for 1-alkyl-3-(1-naphthoyl)indoles at the cannabinoid CB1 and CB2 receptors: steric and electronic effects of naphthoyl substituents. New highly selective CB2 receptor agonists. Bioorg Med Chem 13:89–112

Huffman JW, Padgett LW, Isherwood ML, Wiley JL, Martin BR (2006) 1-Alkyl-2-aryl-4-(1-naphthoyl)pyrroles: new high affinity ligands for the cannabinoid CB1 and CB2 receptors. Bioorg Med Chem Lett 16:5432–5435

Ibsen MS, Connor M, Glass M (2017) Cannabinoid CB1 and CB2 receptor signaling and bias. Cannabis Cannabinoid Res 2:48–60

Jankovics P, Váradi A, Tölgyesi L, Lohner S, Németh-Palotás J, Balla J (2012) Detection and identification of the new potential synthetic cannabinoids 1-pentyl-3-(2-iodobenzoyl)indole and 1-pentyl-3-(1-adamantoyl)indole in seized bulk powders in Hungary. Forensic Sci Int 214:27–32

Järbe TU, Hiltunen AJ, Mechoulam R (1989) Stereospecificity of the discriminative stimulus functions of the dimethylheptyl homologs of 11-hydroxy-delta 8-tetrahydrocannabinol in rats and pigeons. J Pharmacol Exp Ther 250:1000–1005

Johnson MR, Melvin LS (1983) 2-Hydroxy-4-(substituted) phenyl cycloalkanes and derivatives. US Patent 4,371,720

Kikura-Hanajiri R, Uchiyama N, Kawamura M, Goda Y (2013) Changes in the prevalence of synthetic cannabinoids and cathinone derivatives in Japan until early 2012. Forensic Toxicol 31:44–53

Kikura-Hanajiri R, Kawamura NU, Goda Y (2014) Changes in the prevalence of new psychoactive substances before and after the introduction of the generic scheduling of synthetic cannabinoids in Japan. Drug Test Anal 6:832–839

King LA (2014) Legal controls on cannabimimetics: an international dilemma? Drug Test Anal 6:80–87

Lainton JH, Huffman JW, Martin BR, Compton DR (1995) 1-Alkyl-3-(1-naphthoyl)pyrroles: a new class of cannabinoid. Tetrahedron Lett 36:1401–1404

Langer N, Lindigkeit R, Schiebel HM, Ernst L, Beuerle T (2014) Identification and quantification of synthetic cannabinoids in 'spice-like' herbal mixtures: a snapshot of the German situation in the autumn of 2012. Drug Test Anal 6:59–71

Langer N, Lindigkeit R, Schiebel H-M, Papke U, Ernst L, Beuerle T (2016a) Identification and quantification of synthetic cannabinoids in "spice-like" herbal mixtures: update of the German situation for the spring of 2015. Forensic Toxicol 34:94–107

Langer N, Lindigkeit R, Schiebel HM, Papke U, Ernst L, Beuerle T (2016b) Identification and quantification of synthetic cannabinoids in "spice-like" herbal mixtures: update of the German situation for the spring of 2016. Forensic Sci Int 269:31–41

Laprairie RB, Bagher AM, Kelly MEM, Dupré DJ, Denovan-Wright EM (2014) Type 1 cannabinoid receptor ligands display functional selectivity in a cell culture model of striatal medium spiny projection neurons. J Biol Chem 289:24845–24862

Lauckner JE, Hille B, Mackie K (2005) The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB_1 receptor coupling to $G_{q/11}$ G proteins. Proc Natl Acad Sci U S A 102:19144–19149

Leggett JD, Aspley S, Beckett SRG, D'antona AM, Kendall DA, Kendall DA (2004) Oleamide is a selective endogenous agonist of rat and human CB1 cannabinoid receptors. Br J Pharmacol 141:253–262

Lemberger L, Crabtree RE, Rowe HM (1972) 11-Hydroxy-Δ^9-tetrahydrocannabinol: pharmacology, disposition, and metabolism of a major metabolite of marihuana in man. Science 177:62–64

Lichtman AH, Martin BR (2005) Cannabinoid tolerance and dependence. In: Pertwee RG (ed) Cannabinoids. Springer, Berlin, Heidelberg

Little PJ, Compton DR, Mechoulam R, Martin BR (1989) Stereochemical effects of 11-OH-Δ8-THC-dimethylheptyl in mice and dogs. Pharmacol Biochem Behav 32:661–666

Logan BK, Reinhold LE, Xu A, Diamond FX (2012) Identification of synthetic cannabinoids in herbal incense blends in the United States. J Forensic Sci 57:1168–1180

Lovinger DM (2008) Presynaptic modulation by endocannabinoids. Handb Exp Pharmacol 184:435–477

Mackie K (2005) Distribution of cannabinoid receptors in the central and peripheral nervous system. In: Pertwee RG (ed) Cannabinoids. Springer, Berlin, Heidelberg

Mackie K (2008) Cannabinoid receptors: where they are and what they do. J Neuroendocrinol 20:10–14

Makriyannis A, Deng H (2001) Preparation of cannabimimetic indole derivatives with cannabinoid CB1 or CB2 receptor binding affinity. World Patent 2,001,028,557

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561

Mechoulam R, Gaoni Y (1965) A total synthesis of dl-Δ1-tetrahydrocannabinol, the active constituent of hashish1. J Am Chem Soc 87:3273–3275

Mechoulam R, Hanus L (2000) A historical overview of chemical research on cannabinoids. Chem Phys Lipids 108:1–13

Mechoulam R, Shvo Y (1963) Hashish. I. The structure of cannabidiol. Tetrahedron 19:2073–2078

Mechoulam R, Feigenbaum JJ, Lander N, Segal M, Järbe TUC, Hiltunen AJ, Consroe P (1988) Enantiomeric cannabinoids: stereospecificity of psychotropic activity. Experientia 44:762–764

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50:83–90

Morales P, Reggio PH (2017) An update on non-CB1, non-CB2 cannabinoid related G-protein-coupled receptors. Cannabis Cannabinoid Res 2:265–273

Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature 365:61–65

Nakajima J-I, Takahashi M, Seto T, Kanai C, Suzuki J, Yoshida M, Hamano T (2011a) Identification and quantitation of two benzoylindoles AM-694 and (4-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone, and three cannabimimetic naphthoylindoles JWH-210, JWH-122, and JWH-019 as adulterants in illegal products obtained via the internet. Forensic Toxicol 29:95–110

Nakajima JI, Takahashi M, Nonaka R, Seto T, Suzuki J, Yoshida M, Kanai C, Hamano T (2011b) Identification and quantitation of a benzoylindole (2-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl) methanone and a naphthoylindole 1-(5-fluoropentyl-1*H*-indol-3-yl)-(naphthalene-1-yl) methanone (AM-2201) found in illegal products obtained via the internet and their cannabimimetic effects evaluated by in vitro [^{35}S]GTPγS binding assays. Forensic Toxicol 29:132–141

National Research Council (1984) Possible long-term health effects of short-term exposure to chemical agents, volume 2: cholinesterase reactivators, psychochemicals and irritants and vesicants. National Academic Press, Washington DC

Obafemi AI, Kleinschmidt K, Goto C, Fout D (2015) Cluster of acute toxicity from ingestion of synthetic cannabinoid-laced brownies. J Med Toxicol 11:426–429

Pace JM, Tietje K, Dart MJ, Meyer MD (2006) 3-Cycloalkylcarbonylindoles as cannabinoid receptor ligands and their preparation, pharmaceutical compositions and use for treatment of pain. World Patent 2,006,069,196

Pacheco M, Childers SR, Arnold R, Casiano F, Ward SJ (1991) Aminoalkylindoles: actions on specific G-protein-linked receptors. J Pharmacol Exp Ther 257:170–183

Park Y, Lee C, Lee H, Pyo J, Jo J, Lee J, Choi H, Kim S, Hong R, Park Y, Hwang B, Choe S, Jung J (2013) Identification of a new synthetic cannabinoid in a herbal mixture: 1-butyl-3-(2-methoxybenzoyl)indole. Forensic Toxicol 31:187–196

Peace MR, Krakowiak RI, Wolf CE, Poklis A, Poklis JL (2017) Identification of MDMB-FUBINACA in commercially available e-liquid formulations sold for use in electronic cigarettes. Forensic Sci Int 271:92–97

Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA (2010) International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. Pharmacol Rev 62:588–631

Piomelli D (2003) The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4:873–884

Razdan RK (1986) Structure-activity relationships in cannabinoids. Pharmacol Rev 38:75–149

Russell PB, Todd AR, Wilkinson S, Macdonald AD, Woolfe G (1941) 147. Cannabis indica. Part VIII. Further analogues of tetrahydrocannabinol. J Chem Soc:826

Russo EB (2016) Beyond cannabis: plants and the endocannabinoid system. Trends Pharmacol Sci 37:594–605

Seely KA, Patton AL, Moran CL, Womack ML, Prather PL, Fantegrossi WE, Radominska-Pandya A, Endres GW, Channell KB, Smith NH, Mccain KR, James LP, Moran JH (2013) Forensic investigation of K2, spice, and "bath salt" commercial preparations: a three-year study of new designer drug products containing synthetic cannabinoid, stimulant, and hallucinogenic compounds. Forensic Sci Int 233:416–422

Shanks KG, Behonick GS, Dahn T, Terrell A (2013) Identification of novel third-generation synthetic cannabinoids in products by ultra-performance liquid chromatography and time-of-flight mass spectrometry. J Anal Toxicol 37:517–525

Shao Z, Yin J, Chapman K, Grzemska M, Clark L, Wang J, Rosenbaum DM (2016) High-resolution crystal structure of the human CB1 cannabinoid receptor. Nature 540:602

Simolka K, Lindigkeit R, Schiebel H-M, Papke U, Ernst L, Beuerle T (2012) Analysis of synthetic cannabinoids in "spice-like" herbal highs: snapshot of the German market in summer 2011. Anal Bioanal Chem 404:157–171

Smith VJ (2008) Synthesis and pharmacology of N-alkyl-3-(halonaphthoyl)indoles. PhD dissertation, Clemson University

Soethoudt M, Grether U, Fingerle J, Grim TW, Fezza F, De Petrocellis L, Ullmer C, Rothenhäusler B, Perret C, Van Gils N, Finlay D, Macdonald C, Chicca A, Gens MD, Stuart J, De Vries H, Mastrangelo N, Xia L, Alachouzos G, Baggelaar MP, Martella A, Mock ED, Deng H, Heitman LH, Connor M, Di Marzo V, Gertsch J, Lichtman AH, Maccarrone M, Pacher P, Glass M, Van Der Stelt M (2017) Cannabinoid CB2 receptor ligand profiling reveals biased signalling and off-target activity. Nat Commun 8:13958

Szabo B, Schlicker E (2005) Effects of cannabinoids on neurotransmission. In: Pertwee RG (ed) Cannabinoids. Springer, Berlin, Heidelberg

Thakur GA, Tichkule R, Bajaj S, Makriyannis A (2009) Latest advances in cannabinoid receptor agonists. Expert Opin Ther Pat 19:1647–1673

Trachsel D (2012) Fluorine in psychedelic phenethylamines. Drug Test Anal 4:577–590

Trecki J, Gerona RR, Schwartz MD (2015) Synthetic cannabinoid-related illnesses and deaths. N Engl J Med 373:103–107

Turner SE, Williams CM, Iversen L, Whalley BJ (2017) Molecular pharmacology of phytocannabinoids. In: Kinghorn AD, Falk H, Gibbons S, Kobayashi JI (eds) Phytocannabinoids: unraveling the complex chemistry and pharmacology of Cannabis sativa. Springer, Cham

Uchiyama N, Kikura-Hanajiri R, Goda Y (2011) Identification of a novel cannabimimetic phenylacetylindole, cannabipiperidiethanone, as a designer drug in a herbal product and its affinity for cannabinoid CB1 and CB2 receptors. Chem Pharm Bull 59:1203–1205

Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y (2012) Identification of two new-type synthetic cannabinoids, N-(1-adamantyl)-1-pentyl-1*H*-indole-3-carboxamide (APICA) and N-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide (APINACA), and detection of five synthetic cannabinoids, AM-1220, AM-2233, AM-1241, CB-13 (CRA-13), and AM-1248, as designer drugs in illegal products. Forensic Toxicol 30:114–125

Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y (2013a) URB-754: a new class of designer drug and 12 synthetic cannabinoids detected in illegal products. Forensic Sci Int 227:21–32

Uchiyama N, Matsuda S, Kawamura M, Kikura-Hanajiri R, Goda Y (2013b) Two new-type cannabimimetic quinolinyl carboxylates, QUPIC and QUCHIC, two new cannabimimetic carboxamide derivatives, ADB-FUBINACA and ADBICA, and five synthetic cannabinoids detected with a thiophene derivative α-PVT and an opioid receptor agonist AH-7921 identified in illegal products. Forensic Toxicol 31:223–240

United Nations Office on Drugs and Crime, World Drug Report (2017) United Nations publication, Sales No. E.17.XI.6. United Nations Publications, New York

US Drug Enforcement Administration, Office of Diversion Control (2014) National Forensic Laboratory Information System Special Report: Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS 2010–2013. Springfield, VA

Viegas-Junior C, Danuello A, Da Silva Bolzani V, Barreiro EJ, Fraga CA (2007) Molecular hybridization: a useful tool in the design of new drug prototypes. Curr Med Chem 14:1829–1852

Ward SJ, Mastriani D, Casiano F, Arnold R (1990) Pravadoline: profile in isolated tissue preparations. J Pharmacol Exp Ther 255:1230–1239

Weissman A, Milne GM, Melvin LS (1982) Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol. J Pharmacol Exp Ther 223:516–523

Wiley JL, Compton DR, Dai D, Lainton JH, Phillips M, Huffman JW, Martin BR (1998) Structure-activity relationships of indole- and pyrrole-derived cannabinoids. J Pharmacol Exp Ther 285:995–1004

Wiley JL, Smith VJ, Chen J, Martin BR, Huffman JW (2012) Synthesis and pharmacology of 1-alkyl-3-(1-naphthoyl)indoles: steric and electronic effects of 4- and 8-halogenated naphthoyl substituents. Bioorg Med Chem 20:2067–2081

Wiley JL, Marusich JA, Lefever TW, Grabenauer M, Moore KN, Thomas BF (2013) Cannabinoids in disguise: Δ^9-tetrahydrocannabinol-like effects of tetramethylcyclopropyl ketone indoles. Neuropharmacology 75:145–154

Wilkinson SM, Banister SD, Kassiou M (2015) Bioisosteric fluorine in the clandestine design of synthetic cannabinoids. Aust J Chem 68:4–8

Winstock AR, Barratt MJ (2013) Synthetic cannabis: a comparison of patterns of use and effect profile with natural cannabis in a large global sample. Drug Alcohol Depend 136:106–111

Wood TB, Spivey WTN, Easterfield TH (1899) III. – cannabinol. Part I. J Chem Soc Trans 75:20–36

Zuardi AW (2006) History of cannabis as a medicine: a review. Rev Bras Psiquiatr 28:153–157

Zuba D, Byrska B (2013) Analysis of the prevalence and coexistence of synthetic cannabinoids in "herbal high" products in Poland. Forensic Toxicol 31:21–30

The Chemistry and Pharmacology of Synthetic Cannabinoid Receptor Agonist New Psychoactive Substances: Evolution

Samuel D. Banister and Mark Connor

Contents

Abstract

Synthetic cannabinoid receptor agonists (SCRAs) are the largest and most structurally diverse class of new psychoactive substances (NPS). Although the earliest SCRA NPS were simply repurposed from historical academic manuscripts or pharmaceutical patents describing cannabinoid ligands, recent examples bear hallmarks of rational design. SCRA NPS manufacturers have applied traditional

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medicinal chemistry strategies (such as molecular hybridization, bioisosteric replacement, and scaffold hopping) to existing cannabinoid templates in order to generate new molecules that circumvent structure-based legislation. Most SCRAs potently activate cannabinoid type 1 and type 2 receptors (CB_1 and CB_2, respectively), with the former contributing to the psychoactivity of these substances. SCRAs are generally more toxic than the Δ^9-tetrahydrocannabinol (Δ^9-THC) found in cannabis, and this may be due to ligand bias, metabolism, or off-target activity. This chapter will chart the evolution of recently identified SCRA NPS chemotypes, as well as their putative manufacturing by-products and thermolytic degradants, and describe structure-activity relationships within each class.

Keywords

Δ^9-Tetrahydrocannabinol · AB-CHMINACA · AB-FUBINACA · AB-PINACA · AMB-FUBINACA · Cannabinoid · CP 55,940 · JWH-018 · MDMB-CHMICA · NPS · XLR-11

Acronyms of the Discussed New Psychoactive Substances (NPS)

Δ^9-THC	(6a*R*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-dibenzo[*b*,*d*]pyran-1-ol (Δ^9-tetrahydrocannabinol)
3-CAF	Naphthalen-2-yl 1-(2-fluorophenyl)-1*H*-indazole-3-carboxylate
4-HTMPIPO	4-Hydroxy-3,3,4-trimethyl-1-(1-pentyl-1*H*-indol-3-yl)pentan-1-one
5Cl-NNEI	1-(5-Chloropentyl)-*N*-(naphthalen-1-yl)-1*H*-indole-3-carboxamide
5Cl-UR-144	[1-(5-Chloropentyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone
5F-AB-FUPPYCA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(5-fluoropentyl)-5-(4-fluorophenyl)-1*H*-pyrazole-3-carboxamide
5F-AB-PICA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(5-fluoropentyl)-1*H*-indole-3-carboxamide
5F-AB-PINACA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide
5F-ADB-PICA	*N*-[(2*S*)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-(5-fluoropentyl)-1*H*-indole-3-carboxamide
5F-ADB-PINACA	*N*-[(2*S*)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide
5F-AKB-57	Adamantan-1-yl 1-(5-fluoropentyl)-1*H*-indazole-3-carboxylate

5F-AKB-48-7N	*N*-(Adamantan-1-yl)-1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-b]pyridine-3-carboxamide
5F-AMB-PICA	Methyl (2*S*)-2-{[1-(5-fluoropentyl)-1*H*-indole-3-carbonyl]amino}-3-methylbutanoate
5F-AMB-PINACA	Methyl (2*S*)-2-{[1-(5-fluoropentyl)-1*H*-indazole-3-carbonyl]amino}-3-methylbutanoate
5F-AMPPPCA	*N*-(Adamantan-1-yl)-1-(5-fluoropentyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxamide
5F-APICA	*N*-(Adamantan-1-yl)-1-(5-fluoropentyl)-1*H*-indole-3-carboxamide
5F-APINACA	*N*-(Adamantan-1-yl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide
5F-CUMYL-PICA	1-(5-Fluoropentyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indole-3-carboxamide
5F-CUMYL-PINACA	1-(5-Fluoropentyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
5F-MDMB-PICA	Methyl (2*S*)-2-{[1-(5-fluoropentyl)-1*H*-indole-3-carbonyl]amino}-3,3-dimethylbutanoate
5F-MDMB-PINACA	Methyl (2*S*)-2-{[1-(5-fluoropentyl)-1*H*-indazole-3-carbonyl]amino}-3,3-dimethylbutanoate
5F-MN-18	1-(5-Fluoropentyl)-*N*-(naphthalen-1-yl)-1*H*-indazole-3-carboxamide
5F-NNEI	1-(5-Fluoropentyl)-*N*-(naphthalen-1-yl)-1*H*-indole-3-carboxamide
5F-NPB-22-7N	Quinolin-8-yl 1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylate
5F-PB-22	Quinolin-8-yl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate
5F-PCN	1-(5-Fluoropentyl)-*N*-(naphthalen-1-yl)-1*H*-pyrrolo[3,2-*c*]pyridine-3-carboxamide
5F-SBD-005	Naphthalen-1-yl 1-(5-fluoropentyl)-1*H*-indazole-3-carboxylate
A-836339	*N*-(3-(2-methoxyethyl)-4,5-dimethylthiazol-2(3*H*)-ylidene)-2,2,3,3-tetramethylcyclopropane-1-carboxamide
AB-001	(Adamantan-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone
AB-005-azepane	(1-(1-Methylazepan-3-yl)-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone
AB-CHFUPYCA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1*H*-pyrazole-5-carboxamide
AB-CHMINACA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide

AB-CHMINACA-2*H*-indazole	*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-2-(cyclohexylmethyl)-2*H*-indazole-3-carboxamide
AB-FUBICA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1*H*-indole-3-carboxamide
AB-FUBINACA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1*H*-indazole-3-carboxamide
AB-PICA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-pentyl-1*H*-indole-3-carboxamide
AB-PINACA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-pentyl-1*H*-indazole-3-carboxamide
ADB-CHMINACA	*N*-[(2*S*)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide
ADB-FUBICA	*N*-[(2*S*)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1*H*-indole-3-carboxamide
ADB-FUBINACA	*N*-[(2*S*)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1*H*-indazole-3-carboxamide
ADB-PICA	*N*-[(2*S*)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-pentyl-1*H*-indole-3-carboxamide
ADSB-FUB-187	7-Chloro-*N*-[(2*S*)-1-({2-[(cyclopropanesulfonyl)amino]ethyl}amino)-3,3-dimethyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1*H*-indazole-3-carboxamide
APP-FUBINACA	*N*-[(2*S*)-1-Amino-1-oxo-3-phenylpropan-2-yl]-1-[(4-fluorophenyl)methyl]-1*H*-indazole-3-carboxamide
AKB-48	*N*-(Adamantan-1-yl)-1-pentyl-1*H*-indazole-3-carboxamide
AM-2201	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](naphthalen-1-yl)methanone
AMB-CHMICA	Methyl (2*S*)-2-{[1-(cyclohexylmethyl)-1*H*-indole-3-carbonyl]amino}-3-methylbutanoate
AMB-CHMINACA	Methyl (2*S*)-2-{[1-(cyclohexylmethyl)-1*H*-indazole-3-carbonyl]amino}-3-methylbutanoate
AMB-FUBICA	Methyl (2*S*)-2-({1-[(4-fluorophenyl)methyl]-1*H*-indole-3-carbonyl}amino)-3-methylbutanoate
AMB-FUBINACA	Methyl (2*S*)-2-({1-[(4-fluorophenyl)methyl]-1*H*-indazole-3-carbonyl}amino)-3-methylbutanoate
AMB-PICA	Methyl (2*S*)-2-[(1-pentyl-1*H*-indole-3-carbonyl)amino]-3-methylbutanoate

AMB-PINACA	Methyl (2*S*)-2-[(1-pentyl-1*H*-indazole-3-carbonyl)amino]-3-methylbutanoate
AMPPPCA	*N*-(Adamantan-1-yl)-4-methyl-1-pentyl-5-phenyl-1*H*-pyrazole-3-carboxamide
APICA	*N*-(Adamantan-1-yl)-1-pentyl-1*H*-indole-3-carboxamide
APINAC	Adamantan-1-yl 1-pentyl-1*H*-indazole-3-carboxylate
APINACA	*N*-(Adamantan-1-yl)-1-pentyl-1*H*-indazole-3-carboxamide
APINACA-2*H*-indazole	*N*-(Adamantan-1-yl)-2-pentyl-2*H*-indazole-3-carboxamide
BB-22	Quinolin-8-yl 1-(cyclohexylmethyl)-1*H*-indole-3-carboxylate
BIM-018	(Naphthalen-1-yl)(1-pentyl-1*H*-benzimidazol-2-yl)methanone
BiPICANA	*N*-(Naphthalen-1-yl)-1-pentyl-*N*-(1-pentyl-1*H*-indole-3-carbonyl)-1*H*-indole-3-carboxamide
BzODZ-EPyr	3-Benzyl-5-(1-(2-(pyrrolidin-1-yl)ethyl)-1*H*-indol-3-yl)-1,2,4-oxadiazole
CBL-018	Naphthalen-1-yl 1-pentyl-1*H*-indole-3-carboxylate
CBL-2201	Naphthalen-1-yl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate
CP 47,497-C8	2-[(1*S*,3*R*)-3-Hydroxycyclohexyl]-5-(2-methylnonan-2-yl)phenol
CUMYL-4CN-B7AICA	1-(4-Cyanobutyl)-*N*-(2-phenylpropan-2-yl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxamide
CUMYL-5F-P7AICA	1-(5-Fluoropentyl)-*N*-(2-phenylpropan-2-yl)-1*H*-pyrrolo[2,3-b]pyridine-3-carboxamide
CUMYL-BICA	1-Butyl-*N*-(2-phenylpropan-2-yl)-1*H*-indole-3-carboxamide
CUMYL-BINACA	1-Butyl-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
CUMYL-CHMICA	1-(Cyclohexylmethyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indole-3-carboxamide
CUMYL-CHMINACA	1-(Cyclohexylmethyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
CUMYL-FUBICA	1-(4-Fluorobenzyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indole-3-carboxamide
CUMYL-FUBINACA	1-(4-Fluorobenzyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
CUMYL-PEGACLONE	5-Pentyl-2-(2-phenylpropan-2-yl)-2,5-dihydro-1*H*-pyrido[4,3-*b*]indol-1-one

CUMYL-PICA	1-Pentyl-*N*-(2-phenylpropan-2-yl)-1*H*-indole-3-carboxamide
CUMYL-PINACA	1-Pentyl-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
DP-UR-144	(1*H*-Indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone
EG-018	(Naphthalen-1-yl)(9-pentyl-9*H*-carbazol-3-yl)methanone
EG-2201	[9-(5-Fluoropentyl)-9*H*-carbazol-3-yl](naphthalen-1-yl)methanone
FAB-144	[1-(5-Fluoropentyl)-1*H*-indazol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone
FDU-NNEI	1-[(4-Fluorophenyl)methyl]-*N*-(naphthalen-1-yl)-1*H*-indole-3-carboxamide
FDU-PB-22	Naphthalen-1-yl 1-[(4-fluorophenyl)methyl]-1*H*-indole-3-carboxylate
FUB-144	{1-[(4-Fluorophenyl)methyl]-1*H*-indol-3-yl}(2,2,3,3-tetramethylcyclopropyl)methanone
FUB-AKB-48	*N*-(Adamantan-1-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide
FUB-PB-22	Quinolin-8-yl 1-[(4-fluorophenyl)methyl]-1*H*-indole-3-carboxylate
FUBIMINA	[1-(5-Fluoropentyl)-1*H*-benzimidazol-2-yl](naphthalen-1-yl)methanone
JWH-018	(Naphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone
M-5FPIC	Methyl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate
MCBH-1	1-(Cyclohexylmethyl)-2-(4-ethoxybenzyl)-*N*,*N*-diethyl-1*H*-benzo[*d*]imidazole-5-carboxamide
M-CHMIC	Methyl 1-(cyclohexylmethyl)-1*H*-indole-3-carboxylate
MDMB-CHMCZCA	Methyl (2*S*)-2-{[9-(cyclohexylmethyl)-9*H*-carbazole-3-carbonyl]amino}-3,3-dimethylbutanoate
MDMB-CHMICA	Methyl (2*S*)-2-{[1-(cyclohexylmethyl)-1*H*-indole-3-carbonyl]amino}-3,3-dimethylbutanoate
MDMB-CHMINACA	Methyl (2*S*)-2-{[1-(cyclohexylmethyl)-1*H*-indazole-3-carbonyl]amino}-3,3-dimethylbutanoate
MDMB-FUBICA	Methyl (2*S*)-2-({1-[(4-fluorophenyl)methyl]-1*H*-indole-3-carbonyl}amino)-3,3-dimethylbutanoate
MDMB-FUBINACA	Methyl (2*S*)-2-({1-[(4-fluorophenyl)methyl]-1*H*-indazole-3-carbonyl}amino)-3,3-dimethylbutanoate

MDMB-PICA	Methyl (2*S*)-3,3-dimethyl-2-[(1-pentyl-1*H*-indole-3-carbonyl)amino]butanoate
MDMB-PINACA	Methyl (2*S*)-3,3-dimethyl-2-[(1-pentyl-1*H*-indazole-3-carbonyl)amino]butanoate
MEPIRAPIM	(4-Methylpiperazin-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone
MN-001	(1-Pentyl-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone
MN-18	*N*-(Naphthalen-1-yl)-1-pentyl-1*H*-indazole-3-carboxamide
MN-25	7-Methoxy-1-[2-(morpholin-4-yl)ethyl]-N-[(1*S*,2*S*,4*R*)-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-1*H*-indole-3-carboxamide
NMP-7	(9-Pentyl-9*H*-carbazol-3-yl)(piperidin-1-yl)methanone
NNEI	*N*-(Naphthalen-1-yl)-1-pentyl-1*H*-indazole-3-carboxamide
NNEI-2*H*-indazole	*N*-(Naphthalen-1-yl)-2-pentyl-2*H*-indazole-3-carboxamide
NNL-3	1-{[1-(5-Fluoropentyl)-1*H*-pyrrolo[2,3-b]pyridine-3-carbonyl]oxy}-1*H*-benzotriazole
N-Phenyl-SDB-006	1-Pentyl-*N*-phenyl-1*H*-indole-3-carboxamide
Org-28611	(*S*)-(1-(Cyclohexylmethyl)-7-methoxy-1*H*-indol-3-yl)(3,4-dimethylpiperazin-1-yl)methanone
PB-22	Quinolin-8-yl 1-pentyl-1*H*-indole-3-carboxylate
PTI-1	*N*-Ethyl-*N*-{[2-(1-pentyl-1*H*-indol-3-yl)-1,3-thiazol-4-yl]methyl}ethanamine
PTI-2	*N*-(2-Methoxyethyl)-*N*-{[2-(1-pentyl-1*H*-indol-3-yl)-1,3-thiazol-4-yl]methyl}propan-2-amine
QMPSB	Quinolin-8-yl 4-methyl-3-(piperidine-1-sulfonyl)benzoate
RCS-4-*N*-Me	(4-Methoxyphenyl)(1-methyl-1*H*-indol-3-yl)methanone
SBD-005	Naphthalen-1-yl 1-pentyl-1*H*-indazole-3-carboxylate
SBD-006	*N*-Benzyl-1-pentyl-1*H*-indole-3-carboxamide
THJ-018	(Naphthalen-1-yl)(1-pentyl-1*H*-indazol-3-yl)methanone
THJ-2201	[1-(5-Fluoropentyl)-1*H*-indazol-3-yl](naphthalen-1-yl)methanone
TMCP-020	(1-Heptyl-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone
UR-144	(1-Pentyl-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone
XLR-11	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone

1 Introduction

Since the first examples were identified as new psychoactive substances (NPS) 10 years ago, synthetic cannabinoid receptor agonists (SCRAs) have proliferated exponentially. SCRAs, along with synthetic cathinones, are one of the largest classes of NPS and display the greatest structural diversity. SCRA NPS differ chemically and pharmacologically from the major psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**, Fig. 1). Like Δ^9-THC, SCRAs all are intended to activate the cannabinoid type 1 receptor (CB_1) and most also act as agonists of the type 2 receptor (CB_2). The phytocannabinoid-like CP 47,497-C8 (**2**) was detected alongside JWH-018 (**3**) in a seminal report describing the first SCRA NPS in 2008; however, the subsequent structural evolution of this class has tended away from natural product scaffolds. Many SCRAs described by scientists decades prior were discovered as NPS between 2008 and 2011, especially those reported by John Huffman (JWH series, e.g., JWH-018) and Alexandros Makriyannis (AM series, e.g., AM-2201, **4**). However, around 2012, more recent SCRA examples from the pharmaceutical patent literature emerged as NPS. Initially these analogs contained structural variations at the 3-acyl position of JWH-type indoles, typified by the Abbott compound UR-144 (**5**), and adamantane AB-001 (**6**). Shortly thereafter, entirely novel structures featuring non-acyl linkers emerged (see Sect. 3), such as esters (e.g., PB-22, **7**) and amides (e.g., APICA, **8**), as well as scaffold hopping analogs like indazole AKB-48 (**9**).

Despite their chemical heterogeneity, most SCRA NPS are encompassed by a generic Markush structure comprised of four subunits: core (blue), linker (orange), tail (red), and head (green) groups (Fig. 2). Owing to the promiscuity of the CB_1 orthosteric site, many subunit combinations produce viable SCRAs, and emerging SCRA NPS seem to feature often irrational permutations of new and previously

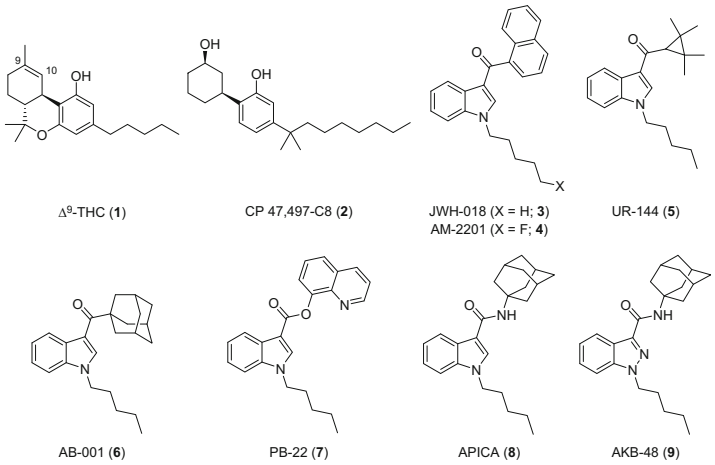


Fig. 1 Representative synthetic cannabinoid receptor agonists identified as new psychoactive substances

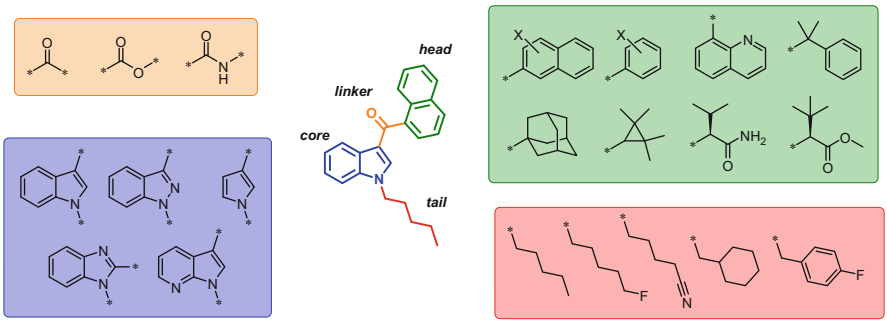


Fig. 2 Generic Markush representation of synthetic cannabinoid receptor agonists obtained by combination of subunits using JWH-018 as a template

identified subunits. Identified aromatic cores are typically indoles or azaindoles (indazole, benzimidazole, pyrrolopyridine), although pyrroles, napthalenes, and thiazoles have been reported. CB_1 has some steric requirements for the tail substituent but otherwise tolerates groups with diverse physicochemical properties including alkyl, alicyclic, heterocyclic, aromatic, and heteroaromatic moieties. The most commonly encountered linkers are ketones, amides, and esters, although simple alkyl tethers and thiazoles have been noted. The greatest diversity is observed for head groups, and these are described more fully in Sects. 2 and 3. Attempts to classify SCRAs will require constant revision as chemical space is expanded (Shevyrin et al. 2016b).

This chapter will review the structure-activity relationships (SARs) of SCRA NPS identified in the past decade, as well as their manufacturing by-products and thermolytic degradants. Binding affinities (K_i) are typically obtained from [^{3}H]CP 55,940 competitive binding experiments in cell membranes expressing human CB_1 or CB_2 (hCB_1, hCB_2). Functional activities (EC_{50}) have usually been derived from fluorescence-based imaging plate reader (FLIPR) experiments in cells stably transfected with hCB_1 or hCB_2 and endogenously expressing G-protein-linked ion channels or agonist-activated ^{35}S-labeled guanosine 5'-O-(3-thiotriphosphate) ([^{35}S]GTPγS) binding in cells or membranes or agonist-inhibited cyclic adenosine monophosphate (cAMP) production assays (Pertwee 2005). It should be noted that variation in reported values occurs due to system differences (radioligand, cell type, etc.), however, such variation tends to be relatively small. For these reasons, K_i and EC_{50} values obtained in the same system are reported wherever possible to enable a direct comparison.

The structural evolution of SCRA NPS has followed identifiable trends; however, it is challenging to capture all of these data in any single review due to the highly dynamic nature of the NPS marketplace and regional variation. We have elected to group SCRAs here by chemotype rather than by chronology.

2 Acylindoles and Acylindazoles

Between 2008 and 2012, almost all detected SCRA NPS were acylindoles related to JWH-018 or AM-2201 and featuring alternative aromatic groups in place of naphthalene (see previous chapter). Following legislative control of such structures in some parts of the world, a number of 3-acylindole SCRA NPS featuring nonaromatic pendant groups appeared, including adamantane derivatives (e.g., AB-001) and tetramethylcyclopropyl (TMCP) analogs (e.g., UR-144). Around the same time, indazole was introduced as a SCRA NPS scaffold to circumvent structure-based prohibition focusing on indoles. Indazoles THJ-018 (**10**, Fig. 3) and THJ-2201 (**11**) have been identified since 2014 in the USA, Japan, and Russia (Diao et al. 2016c; Uchiyama et al. 2014c; Shevyrin et al. 2014). Like their indole analogs, THJ-018 and THJ-2201 were found to be nanomolar CB_1 ligands (K_i = 5.84 and 1.34 nM, respectively) (Hess et al. 2016).

UR-144 was first detected in smoking mixtures in Russia in 2012 (Sobolevsky et al. 2012) and later in Korea (Choi et al. 2013), Japan (Uchiyama et al. 2013a), Germany (Langer et al. 2014), Poland (Zuba et al. 2013), the USA (Shanks et al. 2013), and other countries. UR-144 and analogs featuring the TMCP group were reported by Abbott Laboratories as CB_2-selective SCRAs (Frost et al. 2008, 2010). However, many UR-144 analogs without precedent in the scientific literature have since been identified as CB_1/CB_2 agonists in NPS products, including *N*-heptyl (TMCP-020, **12**) (Shevyrin et al. 2013a), 5-fluoropentyl (XLR-11, **13**) (Choi et al. 2013), 5-chloropentyl (5Cl-UR-144, **14**) (Uchiyama et al. 2013b), and 4-fluorobenzyl (FUB-144, **15**) (Ichikawa et al. 2016) analogs. The indazole analog of XLR-11 has also been reported (FAB-144, **16**) (Hess et al. 2016). In mice,

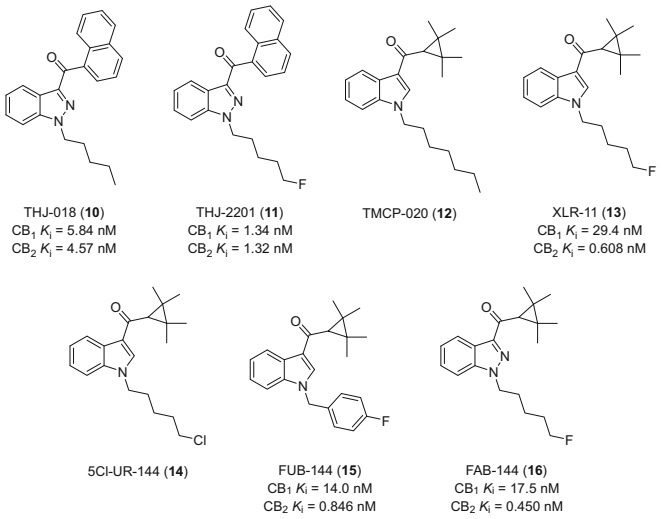


Fig. 3 Acylindoles and acylindazoles identified as SCRA NPS

UR-144 and XLR-11 were found to be more potent cannabimimetic agents than Δ^9-THC, and to substitute for Δ^9-THC in drug discrimination experiments (Wiley et al. 2013), confirming their psychoactivity.

XLR-11 was one of the most prevalent TMCP SCRAs in the early 2010s. Despite appearing in Korea for the first time in 2012, by 2013 XLR-11 was the most frequently seized cannabinoid (Chung et al. 2014). XLR-11 is associated with a large number of adverse reactions and fatalities, and this may be a function of its popularity and/or intrinsic toxicity (Louis et al. 2014; Shanks et al. 2015). XLR-11 was associated with several clusters of acute kidney injury (AKI) (Centers for Disease Control and Prevention 2013; Thornton et al. 2013; Buser et al. 2014), and recent findings suggest this may be due to mechanistic impairment of mitochondrial function in proximal tubule cells (Silva et al. 2018).

3 Linker Modification

Following broad, structure-based prohibition of acylindoles and indazoles in many parts of the world in the early 2010s, SCRA manufacturers began to explore non-acyl linkers including esters (Sect. 3.1), amides (Sect. 3.2), and heteroaromatic bioisosteres of carbonyl linkers (Sect. 3.3). Of these, the amides have remained the most prevalent class and represent the largest number of SCRA NPS identified to date.

3.1 Indole and Indazole Esters

Two of the earliest indole-3-carboxylate SCRA NPS, PB-22 (also known as QUPIC, **7**) and BB-22 (also known as QUCHIC, **17**, Fig. 4), were reported in Japan in 2013, although their design origins are unclear (Uchiyama et al. 2013b). The terminally fluorinated PB-22 analog, 5F-PB-22 (**18**), was identified in 2013 in Russia, Belarus, and Japan (Uchiyama et al. 2014a; Shevyrin et al. 2013b). The 8-hydroxyquinoline group of PB-22, BB-22, and 5F-PB-22 was replaced by the naphthalene subunit of JWH-018 to give esters CBL-018 (**19**) and CBL-2201 (also known as NM-2201, **20**) with retention of CB_1 affinity (Kondrasenko et al. 2015; Hess et al. 2016). The 4-fluorobenzyl analogs of PB-22 and CBL-018, FUB-PB-22 (**21**, $K_i = 0.386$ nM) and FDU-PB-22 (**22**, $K_i = 1.19$ nM), respectively, also demonstrated high affinity for CB_1. Replacing the 4-fluorobenzyl group of FDU-PB-22 with an *N*-(2-fluorophenyl) group (3-CAF, **23**, K_i ~10,000 nM) abolished CB_1 affinity, indicating a minimum steric requirement at this position (Hess et al. 2016).

The indazole analogs of CBL-018 and CBL-2201 have been marketed as SDB-005 (**24**) and 5F-SDB-005 (**25**), not to be confused with structurally distinct indole-3-carboxamide SCRAs of the same code names (Banister et al. 2015b; EMCDDA 2017). Replacement of the naphthyl moiety of SDB-005 and 5F-SDB-005 with an adamantane furnished APINAC (**26**) and 5F-AKB-57 (**27**),

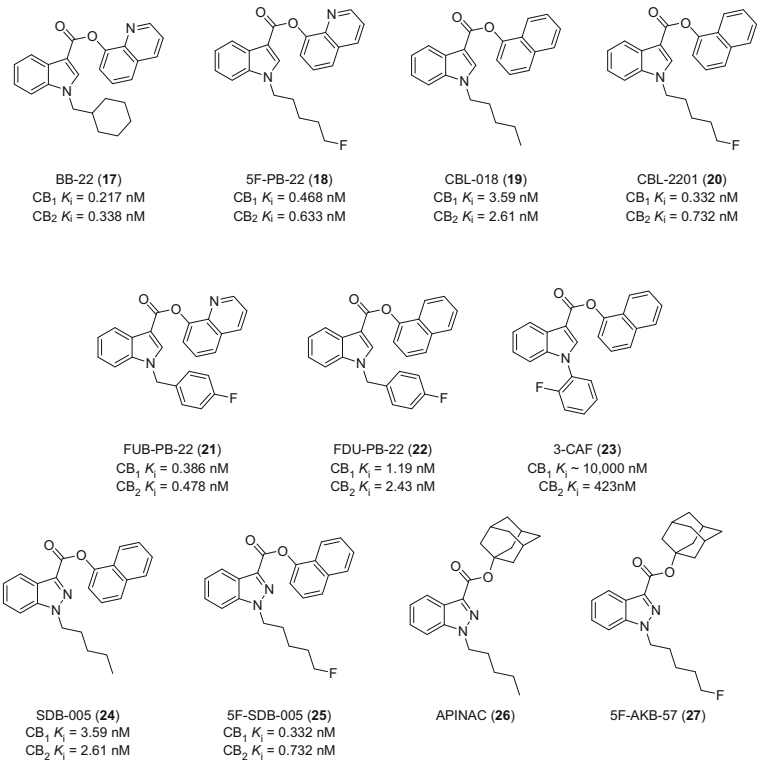


Fig. 4 Indole- and indazole-3-carboxylates identified as SCRA NPS

respectively (Lee et al. 2016; EMCDDA 2017), but nothing is known of their pharmacology.

Indole- and indazole-3-carboxylate SCRAs undergo rapid ester hydrolysis when incubated with rat or human liver microsomes (RLMs and HLMs, respectively), and this has been demonstrated for CBL-2201 ($t_{½}$ = 8.0 min), FUB-PB-22 ($t_{½}$ = 11.5 min), FDU-PB-22 ($t_{½}$ = 12.4 min), and APINAC ($t_{½}$ = 15.2 min) (Diao et al. 2016a, 2017; Savchuk et al. 2017; Hwang et al. 2017). When incubated with human hepatocytes, PB-22 and 5F-PB-22 underwent substantial degradation over 1 h and were undetectable at 3 h, in contrast to the relative stability of SCRAs containing acyl or amido linker (Wohlfarth et al. 2014). Curiously, in vivo pharmacokinetic experiments with APINAC in rats showed much longer half-lives following oral dosing ($t_{½}$ = 3.8 h) or intravenous injection ($t_{½}$ = 11.3 h) (Hwang et al. 2017). In rats, PB-22 and 5F-PB-22 more potently produced a CB_1-mediated hypothermia that was of greater magnitude and duration than other non-ester SCRAs including JWH-018, UR-144, XLR-11, and APICA (Banister et al. 2015b), indicating that metabolism is slower than suggested by in vitro experiments or that potent CB_1 agonist metabolites are formed in rodents.

The quinoline-containing SCRAs may be associated with greater toxicity than other structures in Fig. 4, and several cases of serious adverse events and deaths have been reported. A series of tonic-clonic seizures were reported following use of BB-22, PB-22, and 5F-PB-22, although other JWH-018 analogs were also present (Schep et al. 2015). PB-22 was the cause of poisoning in a man and his dog, and 5F-PB-22 has been quantitatively detected, postmortem, in several cases of fatal intoxication (Angerer et al. 2017; Behonick et al. 2014; Gugelmann et al. 2014).

3.2 Indole and Indazole Amides

Although the design origins of the earliest indole- and indazole-3-carboxylate SCRAs are not known, numerous indole- and indazole-3-carboxamide SCRAs were developed by academic and pharmaceutical research groups in the 2000s (Hynes et al. 2002; Makriyannis and Liu 2003). For example, MN-25 (**28**, Fig. 5) was developed by Bristol-Myers Squibb as an anti-inflammatory agent prior to its discovery on the NPS market (Wrobleski et al. 2003). Org-28611 (**29**) was developed by Organon International as a water-soluble CB_1 agonist for use as an intravenous analgesic and progressed to phase II clinical trials before being abandoned (Adam et al. 2010; Zuurman et al. 2009).

MEPIRAPIM (**30**) was detected in Japan in 2013 and represents a molecular hybrid of JWH-018 and Org-28611; however, its cannabimimetic activity remains to be confirmed (Uchiyama et al. 2014d). Adamantane derivative APICA (2NE1, **31**) and its indazole analogs APINACA (AKB-48, **32**) and 5F-APICA (STS-135, **33**) were discovered in Japan in 2012 (Uchiyama et al. 2012, 2013b; Canazza et al. 2016), with the contemporaneous identification of 5F-APINACA (**34**) (Chung et al. 2014) and a fluorobenzyl analog FUB-AKB-48 (**35**) (Hess et al. 2016).

The amide substituent within this series is not restricted to alicyclic, heterocyclic, or polycarbocyclic groups, and examples featuring an aromatic lipophilic contributor have been reported. During research surrounding SARs of APICA, we reported benzylic analog SDB-006 (**36**), and both SDB-006 and its 5-fluoropentyl analog, 5F-SDB-006 (**37**), were detected in Finland that same year (Banister et al. 2013; EMCDDA 2014). SDB-006, 5F-SDB-006, and *N*-phenyl SDB-006 (**38**) all showed nanomolar binding affinities for CB_1 (K_i = 53–163 nM), although these were an order of magnitude lower than for the adamantane derivatives, suggesting a suboptimal steric contribution from benzyl or phenyl rings (Hess et al. 2016). The 1-naphthyl analog NNEI (**39**, K_i = 1.82 nM), representing a hybrid of JWH-018 and APICA, was first reported by Abbott scientists in 2011 and then in products on the Japanese drug market in 2013 (Blaazer et al. 2011; Uchiyama et al. 2014b). NNEI was detected alongside its 5-fluoropentyl (5F-NNEI, **40**, K_i = 3.69 nM), 5-chloropentyl (5Cl-NNEI, **41**, K_i = 10.2 nM), and indazole (MN-18, **42**, K_i = 3.86 nM) analogs (Shevyrin et al. 2013b; Uchiyama et al. 2014b). The 5-fluoropentyl analog of MN-18, 5F-MN-18 (**43**, K_i = 1.65 nM), was identified in Japan in 2014 and shows greater CB_1 affinity than the des-fluoro congener (Uchiyama et al. 2014c). The 4-fluorobenzyl analog of NNEI, FDU-NNEI (**44**),

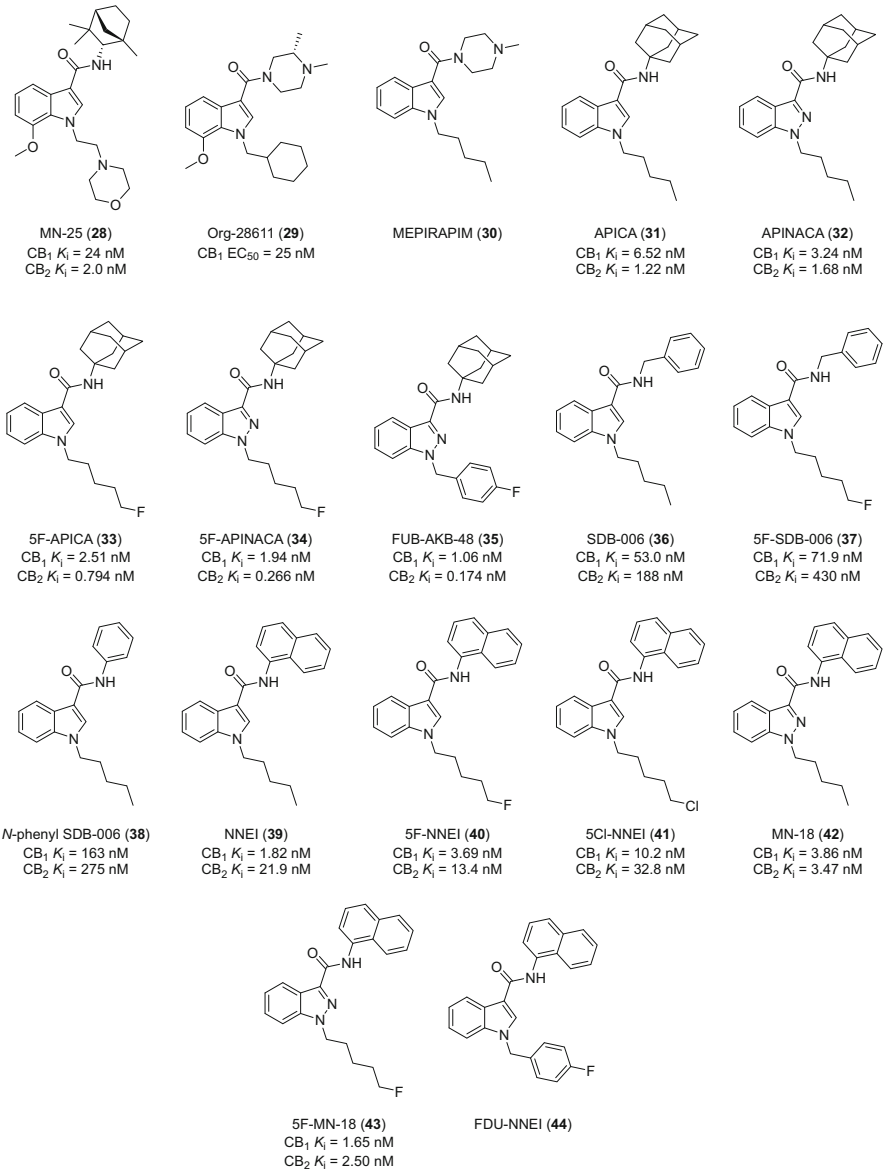


Fig. 5 Indole- and indazole-3-carboxamides identified as SCRA NPS

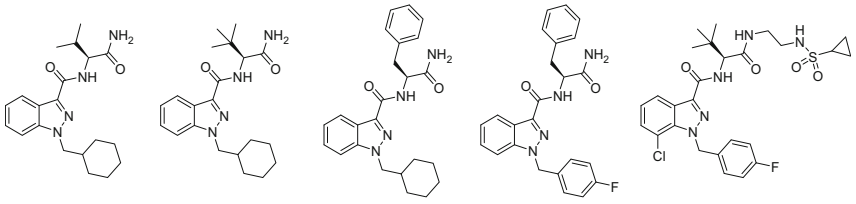
was also detected in Japan, but nothing is currently known of its pharmacology (Uchiyama et al. 2015b).

The incorporation of an aliphatic alkyl chloride in 5Cl-NNEI is unusual, since such haloalkanes can act as alkylating agents in vivo. NNEI analogs would be expected to contain some amount of the 2-naphthyl regioisomers (arising from the

2-naphthylamine always found as a contaminant in 1-naphthylamine), with metabolic liberation of the established carcinogen 2-naphthylamine (Vineis and Pirastu 1997).

3.2.1 Amino Acid Derivatives

Within the broader indole- and indazole-3-carboxamide class, SCRAs featuring pendant amino acid amides or esters are the most prevalent SCRA NPS chemotypes in recent years (**45**–**77**, Figs. 6, 7, and 8). This class is disproportionately associated with serious adverse effects and fatalities; however, it is difficult to delineate intrinsic toxicity from prevalence. An informal and semi-systematic nomenclature has developed around this class. The prefix denotes a pendant valinamide (AB), *tert*-



AB-CHMINACA (**45**)
CB_1 K_i = 0.519 nM
CB_1 EC_{50} = 2.55 nM

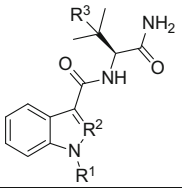
ADB-CHMINACA (**46**)
CB_1 K_i = 0.289 nM
CB_1 EC_{50} = 0.620 nM

APP-CHMINACA (**47**)
CB_1 K_i = 47.6 nM
CB_1 EC_{50} = 2150 nM

APP-FUBINACA (**48**)
CB_1 K_i = 708 nM

ADSB-FUB-187 (**49**)
CB_1 K_i = 0.09 nM
CB_1 EC_{50} = 1.09 nM

Fig. 6 Selected amino acid-derived indole- and indazole-3-carboxamide SCRAs



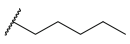
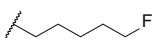
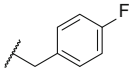
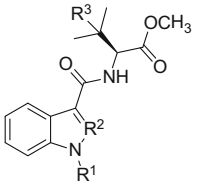
Compound	Name	R^1	R^2	R^3	CB_1 EC_{50} (nM)	CB_2 EC_{50} (nM)
50	AB-PICA		CH	H	12	12
51	ADB-PICA			CH_3	0.69	1.8
52	AB-PINACA		N	H	1.2	2.5
53	ADB-PINACA			CH_3	0.52	0.88
54	5F-AB-PICA		CH	H	5.2	8.9
55	5F-ADB-PICA			CH_3	0.77	1.2
56	5F-AB-PINACA		N	H	0.48	2.6
57	5F-ADB-PINACA			CH_3	0.24	2.1
58	AB-FUBICA		CH	H	21	15
59	ADB-FUBICA			CH_3	2.6	3.0
60	AB-FUBINACA		N	H	1.8	3.2
61	ADB-FUBINACA			CH_3	1.2	3.5

Fig. 7 Valinamide- and *tert*-leucinamide-derived indole- and indazole-3-carboxamide SCRAs



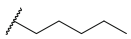
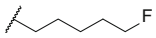
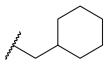
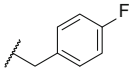
Compound	Name	R^1	R^2	R^3	CB_1 EC_{50} (nM)	CB_2 EC_{50} (nM)
62	AMB-PICA		CH	H	18	23
63	MDMB-PICA			CH_3	1.7	17
64	AMB-PINACA		N	H	3.3	16
65	MDMB-PINACA			CH_3	1.4	28
66	5F-AMB-PICA		CH	H	2.4	4.6
67	5F-MDMB-PICA			CH_3	0.45	7.4
68	5F-AMB-PINACA		N	H	1.9	10
69	5F-MDMB-PINACA			CH_3	0.59	7.5
70	AMB-CHMICA		CH	H	3.5	12
71	MDMB-CHMICA			CH_3	10	71
72	AMB-CHMINACA		N	H	5.1	29
73	MDMB-CHMINACA			CH_3	10	128
74	AMB-FUBICA		CH	H	36	14
75	MDMB-FUBICA			CH_3	2.7	25
76	AMB-FUBINACA		N	H	2.0	18
77	MDMB-FUBINACA			CH_3	3.9	55

Fig. 8 Methyl valinate- and *tert*-leucinate-derived indole- and indazole-3-carboxamide SCRAs

leucinamide (ADB), phenylalaninamide (APP), methyl valinate (AMB), or methyl *tert*-leucinate (MDMB) extending from the amide. In the second part of the name, the *N*-1 substituent is designated *p*entyl (P), 5-*f*luoropentyl (5F, prepended), 4-*flu*oro*b*enzyl (FUB), or *c*yclo*h*exyl*m*ethyl (CHM), attached to an *i*ndole-3-*c*arbox*a*mide (ICA) or *in*d*a*zole-3-*c*arbox*a*mide (INACA) core.

Although seldom falling within specific examples or claims, amino acid-derived SCRA NPS are clearly inspired by a series of patents granted to Bristol-Myers Squibb and Pfizer for the development of analgesics targeting CB_1 (Chen et al. 2002; Buchler et al. 2009a, b). Several amino acid-derived SCRAs explicitly described by Pfizer, including AB-CHMINACA (**45**, K_i = 0.519 nM, EC_{50} = 2.55 nM), ADB-CHMINACA (MAB-CHMINACA, **46**, K_i = 0.289 nM, EC_{50} = 0.620 nM), APP-CHMINACA (PX-3, **47**, K_i = 47.6 nM, EC_{50} = 2,150 nM), APP-FUBINACA (**48**, K_i = 708 nM, EC_{50} not determined), and ADSB-FUB-187 (**49**, K_i = 0.09 nM, EC_{50} = 1.09 nM) were later identified as NPS (Buchler et al. 2009a, b; Uchiyama et al. 2014c; Wurita et al. 2015; Langer et al. 2016). Although

these patents focused extensively on SAR involving substituents around the indazole ring and on the terminal amide, ADSB-FUB-187 is one of the only SCRA NPS identified to date that includes such features. Almost all other examples have featured characteristic groups at the 1-position of the indole/indazole core, and valinamide or *tert*-leucinamide pendant groups (**50–61**, Fig. 7). Excluding AB-FUBINACA and ADB-FUBINACA, these SCRAs are not found in the Pfizer patents and were unknown prior to their identification on the NPS market.

The systematic investigation of all indole- and indazole-3-carboxamides featuring a pentyl, 5-fluoropentyl, or 4-fluorobenzyl group at the 1-position and valinamide or *tert*-leucinamide side chains (**50–61**) in CB_1 and CB_2 FLIPR assays revealed notable SARs for this class (Banister et al. 2015a). Compounds **50–61** were all nanomolar CB_1 agonists (EC_{50} = 0.24–21 nM), and *N*-1 substituent (R^1) had little effect on overall potency. However, within each congeneric series comprising the same *N*-1 group, indazoles (R^2 = N) with a valinamide group (R^3 = H) showed potency roughly an order of magnitude greater than the corresponding indoles (R^2 = CH). A smaller potency increase was seen for the analogous *tert*-leucinamide (R^3 = CH_3) comparison.

Following the identification and control of **50–61** in the mid-2010s, a second iteration of this class of SCRAs appeared with head groups featuring the methyl esters of valinate and *tert*-leucinate (**62–77**, Fig. 8). Subjecting this class to the same CB_1 and CB_2 FLIPR assays described above demonstrated that all ligands were potent CB_1 agonists (EC_{50} = 0.45–36 nM) and SAR trends were consistent with the valinamide and *tert*-leucinamide series (Banister et al. 2016). Generally, several chemically distinct *N*-1 substituents (R^1) were tolerated, indazoles (R^2 = N) were more potent than indoles (R^2 = CH), and *tert*-leucinates (R^3 = CH_3) were more potent than valinates (R^3 = H).

Many SCRA NPS are achiral; however, amino acid-derived SCRAs contain an asymmetric carbon atom. The Pfizer patents include only (*S*)-stereoisomers of SCRAs, derived from the abundant and inexpensive L-amino acids with retention of stereochemistry. Enantiomeric resolution of 11 herbal samples containing 5F-AB-PINACA and 5F-AMB-PINACA (also known as 5F-AMB) showed that all samples analyzed contained the (*S*)-enantiomers, with the (*R*)-enantiomers detected as less than 20% of two samples only (Doi et al. 2016), and the absolute configuration of seized MDMB-CHMICA was also (*S*) in a separate study (Andernach et al. 2016). The CB_1 agonist activities of APP-CHMINACA, MDMB-FUBICA, and related analogs were shown to be greater for (*S*)- than for (*R*)-enantiomers in all cases, although the *S*/*R* potency ratios ranged from 5 to over 3,000 between specific enantiomeric pairs (Doi et al. 2018).

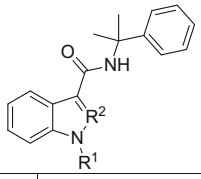
Amino acid-derived SCRAs shown in Figs. 6, 7, and 8 have been associated with the largest and most severe mass casualty events in the USA, suggesting chemotypic clustering of SCRA toxicity (Monte et al. 2014; Trecki et al. 2015; Tyndall et al. 2015; Springer et al. 2016; Adams et al. 2017). Compared to earlier generations of SCRAs, disproportionate numbers of fatal intoxications have been reported for AB-PINACA, AB-CHMINACA, AB-FUBINACA, ADB-FUBINACA, 5F-AMB, 5F-ADB (5F-MDMB-PINACA), MDMB-FUBINACA, and MDMB-CHMICA

(Adamowicz 2016; Shanks et al. 2016; Backberg et al. 2017; Usui et al. 2018). The occupational exposure of law enforcement agents to AB-PINACA during a raid of an illegal laboratory highlights the potency of such compounds (Tapp et al. 2017).

3.2.2 Cumylamine Derivatives

Benzylic indole- and indazole-3-carboxamide SCRAs (e.g., **36** and **37**) generally demonstrate reduced CB_1 affinity compared to larger aromatic and nonaromatic groups. The exception to this trend is a series of cumylamine derivatives (e.g., **78**–**87**, Fig. 9) intended to treat pain and nausea, stimulate appetite, and enhance mood, as reported in a patent in 2014 (Bowden and Williamson 2014). Rate enhancement of intramolecular reactions in substrates containing judicious gem-dimethyl groups has long been appreciated in synthetic organic chemistry (the Thorpe-Ingold effect), and the utility of such groups to control conformation in medicinal chemistry is increasingly apparent (Jung and Piizzi 2005; Talele 2018). CUMYL-PICA (**80**, EC_{50} = 4.2 nM), the gem-dimethyl analog of SDB-006 (**36**, EC_{50} = 115 nM), shows more than 20 times the potency as a CB_1 agonist (Banister et al. 2015b; Longworth et al. 2017a).

Most SCRA NPS are crystalline solids; however, many cumylamine-derived SCRAs are viscous, sticky oils. There is a single case report of occupational transdermal exposure to CUMYL-PINACA in several Slovenian custom inspectors who seized a package imported from Hong Kong (Dobaja et al. 2017).



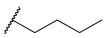
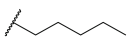
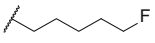
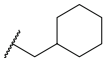
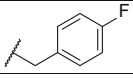
Compound	Synonyms	R^1	R^2	CB_1 EC_{50} (nM)	CB_2 EC_{50} (nM)
78	CUMYL-BICA		CH	8.0	24.5
79	CUMYL-BINACA		N	1.6	12.0
80	CUMYL-PICA		CH	4.2	58.4
81	CUMYL-PINACA		N	2.3	107
82	5F-CUMYL-PICA		CH	2.8	39.6
83	5F-CUMYL-PINACA		N	0.43	11.3
84	CUMYL-CHMICA		CH	9.5	122
85	CUMYL-CHMINACA		N	1.7	90.0
86	CUMYL-FUBICA		CH	12.3	45.5
87	CUMYL-FUBINACA		N	1.8	23.7

Fig. 9 Cumylamine-derived indole- and indazole-3-carboxamide SCRAs

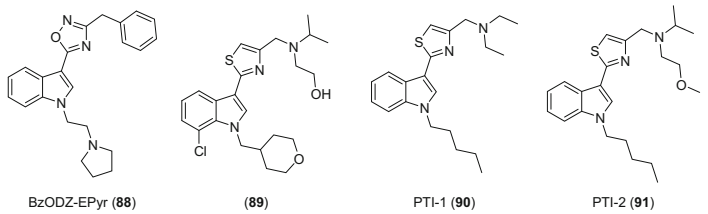


Fig. 10 Oxadiazoles and thiazoles identified as SCRA NPS

3.3 Heteroaromatic Bioisosteres of Ester and Amides Linkers

Small heteroaromatic rings like oxadiazoles and thiazoles are sometimes used as bioisosteres of esters and amides (Patani and Lavoie 1996), and numerous SCRAs featuring heterocyclic linkers have been developed in the past decade. The 1,2,4-oxadiazole BzODZ-EPyr (**88**, Fig. 10) was developed as a novel cannabinoid analgesic by Amrad in 2004 and was one of the most active in the series in a mouse vas deferens bioassay (Moloney and Robertson 2002; Moloney et al. 2008), before it appeared briefly in the Russian SCRA NPS market (Shevyrin et al. 2016a).

In the 2000s, Organon Laboratories explored SCRA thiazole indoles bearing (tetrahydropyran-4-yl)methyl or (cyclohexyl)methyl substituents at the indole nitrogen (Adam-Worrall et al. 2005; Adam 2008). Following acquisition by Schering-Plough and a merger with Merck, Merck extensively explored CB_1 agonists featuring oxazole, thiazole, 1,2,4- and 1,3,4-oxadiazole, and 1,2,4-dithiazole heterocyclic linkers (Morrison et al. 2011). Ultimately, Merck selected **89** ($EC_{50} = 10$ nM) for clinical development based on its long plasma half-life, brain penetration, and strong analgesia (Ratcliffe et al. 2011). Several years later in 2014, two thiazole indoles, PTI-1 (**90**) and PTI-2 (**91**), were seized by German custom authorities in a shipment from China (Westphal et al. 2015). PTI-1 and PTI-2 can be considered molecular hybrids of reported Organon compounds featuring the pentyl chain of JWH-018 rather than an ali- or heterocyclic indole substituent. Like BzODZ-EPyr, PTI-1 and PTI-2 seem to have enjoyed a short-lived existence in the NPS marketplace, and nothing is known of their pharmacology.

4 Scaffold Hopping

As increasingly large swathes of chemical space surrounding indole and indazole SCRA NPS are subjected to control measures, SCRA manufacturers have exploited the well-known medicinal chemistry technique of scaffold hopping to circumvent legislative changes. In drug discovery, scaffold hopping aims to discover structurally novel chemical entities by altering the central core of a known bioactive lead molecule (Böhm et al. 2004). In the case of SCRA NPS, the intention is to retain

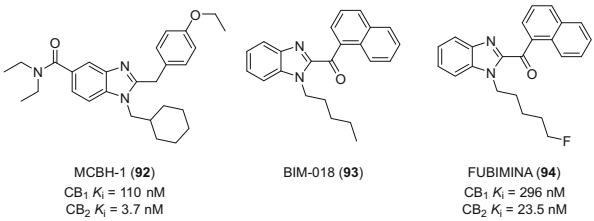


Fig. 11 Benzimidazoles identified as SCRA NPS

CB_1 agonist activity by creating a new compound that falls outside the definitional limits of structure-based legislation.

4.1 Benzimidazoles

A library of CB_2-selective agonists based on a benzimidazole was developed by AstraZeneca, and although nearly all had negligible CB_1 affinity (micromolar), MCBH-1 (**92**, $K_i = 110$ nM, Fig. 11) had the highest CB_1 affinity in the series (Pagé et al. 2008). This is likely the reason MCBH-1 was identified by German custom authorities in 2014 (Westphal et al. 2015). Scaffold hopping analogs of JWH-018 and AM-2201 featuring a 2-naphthoylbenzimidazole in place of the 3-naphthoylindole, BIM-018 (**93**) and FUBIMINA (BIM-2201, **94**), respectively, have also been detected in Japan, Russia, and the USA (Uchiyama et al. 2014d; Shevyrin et al. 2014; Diao et al. 2016b). Limited pharmacological data are available for SCRAs like **93** and **94**, but FUBIMINA was shown to be a moderate affinity CB_1 agonist ($K_i = 296$ nM, $EC_{50} = 2{,}466$ nM); 2–3 orders of magnitude less potent than AB-CHMINACA and AB-PINACA in the same assays (Wiley et al. 2015). Additionally, it did not fully substitute for Δ^9-THC in rodent drug discrimination studies (Wiley et al. 2015).

4.2 Azaindoles

Along with indole, indazole is probably the scaffold most widely employed by SCRA NPS manufacturers. However, other [5,6] aromatic systems containing two nitrogen atoms (azaindoles) can be considered scaffold hops from indazole (2-azaindole). Unlike the corresponding benzimidazoles, 1,3-disubstitution of such systems maintains relative spatial orientation of key pharmacophoric groups. In this way, scaffold hopping SCRA NPS are possible with 4-, 5-, 6-, and 7-azaindole systems; pyrrolo[3,2-*b*]pyridine, pyrrolo[3,2-*c*]pyridine, pyrrolo[2,3-*b*]pyridine, and pyrrolo[2,3-*b*]pyridine, respectively.

Several 7-azaindole-3-carboxamide ("7AICA") and 7-azaindole-3-carboxylate SCRA NPS have been reported recently, including 5F-NPB-22-7N (**95**, Fig. 12), 5F-AKB-48-7N (**96**), CUMYL-5F-P7AICA (**97**), and CUMYL-4CN-B7AICA (**98**); however, their pharmacology has not yet been elucidated (Bovens et al. 2017; Liu

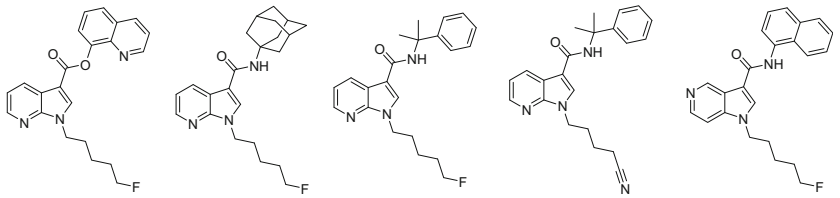


Fig. 12 Azaindoles identified as SCRA NPS

et al. 2017). The 5-azaindole-3-carboxamide 5F-PCN (**99**) was identified in 2014, but it is known how its CB_1 activity compares to 5F-NNEI or 5F-MN-18 (EMCDDA 2016).

4.3 Carbazoles and γ-Carbolines

Academic researchers first reported tricyclic SCRAs based on carbazole and carboline scaffolds, such as CB_1 agonist NMP-7 (**100**, Fig. 13, K_i = 96.9 nM), in 2011 as they sought peripherally restricted CB_2-selective agonists for the treatment of pain (You et al. 2011; Petrov et al. 2013). Elaboration of SARs showed that small amide substituents at the 3-position were preferred for CB_1 binding, with the 9-alkyl group influencing affinity. Acyl substituents at the 3-position, as in **101** (K_i = 291 nM), generally diminished CB_1 affinity, making the discovery of EG-018 (**102**) and EG-2201 (**103**) on the NPS market in 2016 surprising (Liu et al. 2017; EMCDDA 2017). EG-018 and EG-2201 are the 9*H*-carbazole analogs of JWH-018 and AM-2201, respectively, but their CB_1 activity has not been confirmed. MDMB-CHMCZCA is the 9*H*-carbazole analog of MDMB-CHMICA and was identified in SCRA NPS in 2015 (EMCDDA 2016; Weber et al. 2016). The cannabimimetic activity of MDMB-CHMCZCA has not been explored, but is likely based on the SAR trends for related carbazoles.

The γ-carboline CUMYL-PEGACLONE (**105**, K_i = 1.37 nM) was identified along with MDMB-CHMCZCA in Germany immediately following structure-based control of indole, indazole, and benzimidazole SCRAs in 2016 (Angerer et al. 2018). CUMYL-PEGACLONE can be viewed as a carboline analog of CUMYL-PICA in which the amide nitrogen is constrained through an ethene bridge to the 2-position of the indole. Like CUMYL-PICA, CUMYL-PEGACLONE was confirmed as a nanomolar CB_1 agonist (Angerer et al. 2018). The rationale for development of CUMYL-PEGACLONE was likely inspired by the use of γ-carboline to constrain amides in a series of SCRAs reported by Bristol-Myers Squibb in 2003 (Wrobleski et al. 2003).

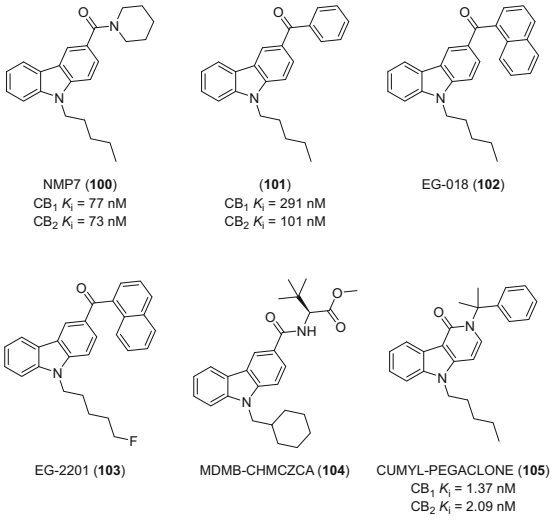


Fig. 13 Carbazoles and carbolines identified as SCRA NPS

5 Miscellaneous Structures

Several miscellaneous SCRAs not conforming to any of the described structural classifications have been reported in NPS products, including A-836339 (**106**, Fig. 14) (Uemura et al. 2014), QMPSB (**107**) (Blakey et al. 2016), AB-CHFUPYCA (3,5-AB-CHMFUPPYCA) (**108**) (Uchiyama et al. 2015a; Mclaughlin et al. 2016), 5F-AB-FUPPYCA (**109**) (Girreser et al. 2016), AMPPPCA (**110**), and 5F-AMPPPCA (**111**) (Jia et al. 2017). A-836339 was developed by Abbott Laboratories as a CB_2-selective agonist for pain, and A-836339 showed typical CB_1-mediated effects in mice at higher doses (Yao et al. 2009). QMPSB belongs to a structurally unique class of arylsulfonamides described as potent SCRAs by Union Chimique Belge (UCB) in 2007 (Lambeng et al. 2007). The remaining pyrazoles bear superficial similarity to 5-aryl-4,5-dihydro-(1*H*)-pyrazoles (pyrazolines) disclosed by Solvay (e.g., **112** and **113**) (Yildirim et al. 2009; Lange et al. 2010). However, minor structural changes are known to convert pyrazoles and dihydropyrazoles to CB_1 antagonists, and the agonist activity of many of these putative SCRAs is yet to be confirmed.

Several fatty acid amide hydrolase (FAAH) inhibitors and endocannabinoid uptake inhibitors have been discovered in NPS products and are presumably intended to produce cannabimimetic effects by preventing the reuptake and degradation of endocannabinoids, respectively, but their cannabimimetic effects in humans have not been confirmed (Nakajima et al. 2013; Uchiyama et al. 2014b).

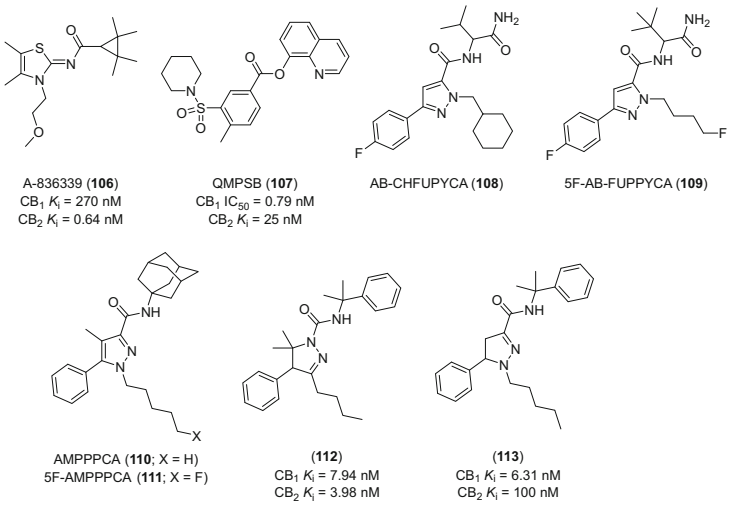


Fig. 14 Miscellaneous structures identified in putative SCRA NPS products

6 Manufacturing By-Products

As with other NPS, positional isomers are commonly encountered in SCRAs, with expected differences in potency, metabolism, and toxicity. Constitutional isomers are likely intended to improve potency or to obfuscate detection. However, as with other illicit drugs, SCRAs are produced with lower quality control than pharmaceuticals. In some cases, putative SCRAs identified in NPS products may, in fact, be manufacturing by-products.

For SCRAs containing an *N*-methylpiperidine substituent, such as AM-1220, AM-2233, and AB-005, the isomeric azepanes have also been detected (**114**, **115**, and **116**, respectively, Fig. 15). The azepane isomer of AM-1220 (**114**) was detected in SCRA products containing AM-1220 in a 30:70 ratio (Kneisel et al. 2012; Nakajima et al. 2013). Indeed, based on the reported synthetic methods for AM-1220, it is feasible that isomeric piperidine and azepane intermediates could be formed following thermally induced partial conversion of alkylating reactant to a rearrangement product (D'ambra et al. 1996; Willis et al. 2005). Similarly, the TMCP derivative AB-005 was detected in Russia and Belarus along with its azepane isomer (**116**) (Shevyrin et al. 2013a). The pharmacology of isomeric azepanes has not been thoroughly explored, however, AM-1220 azepane was found to possess weak cannabimimetic activity in a [^{35}S]GTPγS binding assay (EC_{50} = 7.13 μM) (Nakajima et al. 2014).

The des-pentyl analog of UR-144 (DP-UR-144, **117**) was identified in NPS products in Japan and shown to exert agonist activity at hCB_1 (EC_{50} = 2.36 μM), but nanomolar potency at hCB_2 (EC_{50} = 27.9 nM) (Ichikawa et al. 2016). The synthetic route to UR-144 and its analogs proceeds via alkylation of 2,2,3,3-tetramethylcyclopropanoylindole, so DP-UR-144 is likely an impurity resulting

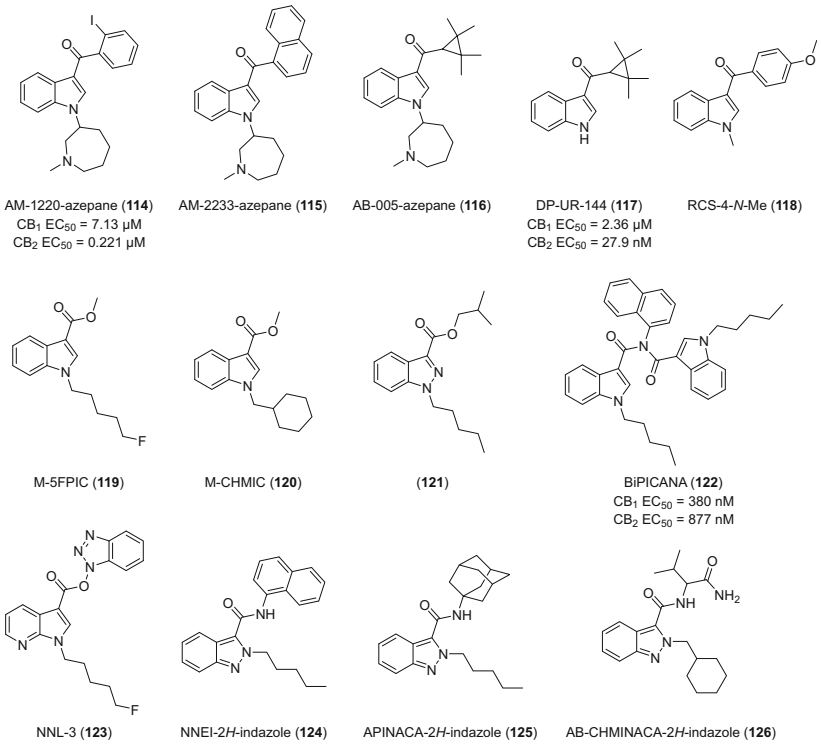


Fig. 15 Manufacturing by-products detected in SCRA NPS products

from incomplete conversion of starting material (Banister et al. 2015b). The *N*-methyl analog of RCS-4 (RCS-4-*N*-Me, **118**) was detected in a German NPS product, although it is unclear how this contaminant might arise as a synthetic impurity. No pharmacological data have been reported for RCS-4-*N*-Me, but it is unlikely to function as a CB_1 agonist with a truncated group replacing the optimal C4-C6 alkyl chain of efficacious benzoylindole SCRAs (Simolka et al. 2012).

A number of 1-substituted indole- and indazole-3-carboxylic esters have been detected in NPS products, albeit in low concentrations, and are probable manufacturing by-products. Methyl esters M-5FPIC (**119**) and M-CHMIC (**120**) have been reported by EU member states, and isobutyl ester **121** was found in Japan (Uchiyama et al. 2015b; EMCDDA 2016). M-CHMIC and M-5FPIC are likely intermediates for the synthesis of MDMB-CHMICA and 5F-MDMB-PICA, respectively, and **121** is thought to result from attempted NNEI synthesis via coupling of the precursor carboxylic acid and an isobutyl carbamate of 8-aminoquinoline.

BiPICANA (**122**) was identified as a dimeric analog of NNEI and is an impurity arising from the addition of two indole acid chlorides to a single aminonaphthalene group, and its structure was confirmed synthetically by using a stoichiometric excess of acid chloride (Nakajima et al. 2014). Despite its unusual structure, BiPICANA demonstrated moderate potency cannabimimetic activity in a [^{35}S]GTPγS binding

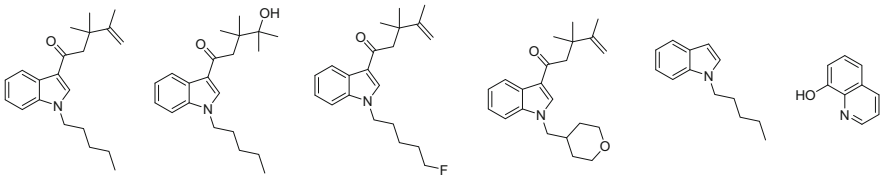
assay (hCB_1 EC_{50} = 380 nM; hCB_2 EC_{50} = 877 nM), highlighting the tolerance of the CB_1 for bulky substituents at the 3-position of the indole-amide chemotype. Similarly, NNL-3 (**123**) is likely a manufacturing by-product due to use of hydroxybenzotriazole (HOBt) as a catalyst and racemization inhibitor in the synthesis of corresponding amides like 5F-AKB-48-7N (Liu et al. 2017). Although it is likely cannabimimetic, the pharmacology of NNL-3 has not been reported.

Several 2*H*-indazole regioisomers of 1*H*-indazole SCRAs have been identified, including NNEI-2*H*-indazole (**124**), APINACA-2*H*-indazole (**125**), and AB-CHMINACA-2*H*-indazole (**126**) (Uchiyama et al. 2015b; Jia et al. 2017). 2*H*-Indazole regioisomers contain the same spatial arrangement of pharmacophoric groups as the benzimidazoles discussed in Sect. 4.1, and it is possible that these regioisomers were intended as scaffold hopping SCRAs. However, the first step in the synthesis of AB-CHMINACA and related 1,3-disubstituted indazoles is alkylation of indazole-3-carboxylate ester to produce 1-alkyl-1*H*-indazole and 2-alkyl-2*H*-indazole intermediates, with regioselectivity primarily determined by choice of base (Longworth et al. 2016). The former intermediate proceeds to give 1-alkyl-1*H*-indazole SCRAs, while subjecting the latter to the same subsequent steps gives 2-alkyl-2*H*-indazole regioisomers (Longworth et al. 2016). Several 2-alkyl-2*H*-indazole isomers were found to possess micromolar potency as $CB_1$and CB_2 agonists when compared to the corresponding 1-alkyl-1*H*-indazole SCRAs in a FLIPR membrane potential assay.

7 Thermolytic Degradants

The common routes of administration for most classes of NPS are ingestion or insufflation; however, since SCRAs are most commonly found in smoking mixtures, in vivo SCRA pharmacology and toxicology are confounded by the formation of pyro- and thermolytic degradants (Bell and Nida 2015). Some SCRAs, such as PB-22, are sufficiently unstable that they undergo thermal degradation during analysis by GC-MS (Tsujikawa et al. 2013). The choice of solvents and the occurrence of transesterification reactions are therefore important considerations under such instrumental conditions.

Several ring-opened degradants of UR-144 (e.g. **127** and **128**, Fig. 16) have been confirmed in controlled pyrolysis experiments and clinical toxicology cases



(**127**)
CB_1 K_i = 5.62 nM
CB_2 K_i = 1.81 nM

4-HTMPIPO (**128**)

(**129**)
CB_1 K_i = 2.84 nM
CB_2 K_i = 1.03 nM

(**130**)
CB_1 K_i = 2.30 nM
CB_2 K_i = 0.71 nM

(**131**)

(**132**)

Fig. 16 Confirmed thermolytic degradants of SCRA NPS

(Adamowicz et al. 2013; Grigoryev et al. 2013; Kavanagh et al. 2013). A UR-144 degradant (**127**, EC_{50} = 111 nM) was more potent as a CB_1 agonist than UR-144 itself (EC_{50} = 401 nM) and, when compared to an equivalent dose of UR-144 in mice (15 mg/kg, i.p.), induced a greater degree of hypothermia and catalepsy suggesting that it is also more potent in vivo (Kaizaki-Mitsumoto et al. 2017).

The generality of thermal ring opening in TMCP SCRAs was recently described, and degradants **127**, **129**, and **130** were all higher affinity CB_1 ligands (K_i = 2.30–5.62 nM) than the parent SCRAs UR-144, XLR-11, and A-834,735 (K_i = 18.3–25.9 nM), respectively (Thomas et al. 2017). In the same study, PB-22 underwent thermal decarboxylation to yield *N*-pentylindole (**131**) and 8-hydroxyquinoline (**132**), both of which were inactive in cannabinoid assays (K_i > 10 μM).

8 Toxicity

SCRAs are significantly more toxic than cannabis or Δ^9-THC; deaths from cannabis are exceedingly rare (Hartung et al. 2014). In contrast to cannabis, SCRA use is associated with serious adverse events including myocardial infarction, ischemic stroke, acute kidney injury (AKI), seizures, and psychiatric complications (Tait et al. 2016; Babi et al. 2017; Lamy et al. 2017). Clinical confirmation of SCRAs in toxicology case reports provides insight into the prevalence and toxicity of specific compounds; however, these data are dually confounded by the selection biases and underrepresentation associated with screening novel drugs in a challenging patient population. Additionally, linking characteristic toxidromes to specific SCRAs can be challenging since NPS products often contain multiple substances, poor quality control, and their consumers are frequently polysubstance users.

SCRA toxicity is not uniform across all structural classes and does not appear to be a strict function of binding affinity or potency at CB_1, and it is possible – if unlikely – that toxicity differences are reflective of SCRA prevalence. SCRA toxicity is more likely a function of intrinsic activity, ligand bias (functional selectivity), activity of metabolites or degradants, or off-target (non-cannabinoid) activity of SCRAs, metabolites, or degradants. As discussed in the previous chapter, signaling bias does not appear to be a prominent feature of SCRAs to date, but more systematic investigation is needed. In contrast to Δ^9-THC, which is largely rendered inactive through oxidative metabolism (Adams and Martin 1996), many SCRAs produce metabolites with differing pharmacological profiles at CB_1 and CB_2 receptors (Brents et al. 2011; Chimalakonda et al. 2012; Rajasekaran et al. 2013; Longworth et al. 2017b; Tai and Fantegrossi 2017).

Very little is known about the off-target activity of SCRAs. Interactions with 5-HT (2B, 2C, and 6), muscarinic acetylcholine (M1), $GABA_A$, and GPR55 receptors have been reported for some examples (Hess et al. 2016; Wiley et al. 2016). Several SCRAs were efficacious inhibitors of the cardiotoxic hERG channel, albeit with micromolar potencies (Wiley et al. 2016). Beyond any off-target activity of SCRAs themselves, there exists the possibility that some adverse effects are due to

agonist-directed CB_1 interactions with other neurotransmitter systems (Fantegrossi et al. 2018). For example, several SCRAs have been shown to induce hypertensive effects through non-cannabinoid mechanisms, and this may contribute to their toxicity (Schindler et al. 2017).

There are limited preclinical data comparing the acute and chronic effects of SCRAs to each other and to Δ^9-THC (Castaneto et al. 2014; Cohen and Weinstein 2018; Schreiber et al. 2018). Centrally active SCRAs dose dependently exert CB_1-mediated hypothermic, bradycardic, hypolocomotive, and analgesic effects in rodents and typically substitute for Δ^9-THC (Gatch and Forster 2014, 2015, 2016, 2018; Kevin et al. 2017; Gamage et al. 2018). Beyond differences in potency, there is emerging evidence that SCRAs produce dissimilar phenotypic profiles in rodents (Maeda et al. 2018).

Confirming the dependence liability of SCRAs by exploring their reinforcing effects in rodents is challenging (Zanda and Fattore 2018). In humans, anecdotal reports suggest that some SCRAs show potential for dependence and produce a severe and characteristic withdrawal syndrome that differs from cannabis withdrawal (Nacca et al. 2013; Van Hout and Hearne 2016). Formalized studies of the effects of early generations of SCRAs, such as JWH-018, in humans are underway and will undoubtedly offer new perspectives on the psychopharmacology of SCRAs in users (Theunissen et al. 2018).

9 Conclusions

Data regarding the prevalence and marketplace lifetimes of SCRA NPS is incomplete. Trends in the SCRA marketplace must be inferred from law enforcement seizures, physician case reports, and clinical toxicologists performing routine analytical confirmation of these substances. The contract manufacture of SCRAs is based on a relatively flat pricing structure per unit of raw material, with obvious financial incentive to produce the most potent CB_1 agonists for maximal dilution in consumer NPS products, and the structural evolution of SCRA NPS has tended toward increasingly potent molecules (Surmont et al. 2017).

Since the identification of the first two SCRAs in NPS products in 2008, hundreds of new SCRAs have emerged; some repurposed from published chemical literature and others rationally designed by anonymous chemists. SCRA manufacturers have innovated in response to a shifting regulatory landscape by utilizing the same methods employed by the pharmaceutical industry to design drugs: bioisosteric replacement, molecular hybridization, scaffold hopping, and other techniques. Historically, a latency of several years exists between the disclosure of new cannabinoid chemotypes in the scientific literature and their emergence as SCRA NPS. The SCRA NPS of the future are likely to be somewhat predictable derivatives of potent CB_1 agonists currently being prepared by researchers in the cannabinoid field.

References

Adam J (2008) Indole derivatives. World Patent 2,008,101,995

Adam JM, Cairns J, Caulfield W, Cowley P, Cumming I, Easson M, Edwards D, Ferguson M, Goodwin R, Jeremiah F, Kiyoi T, Mistry A, Moir E, Morphy R, Tierney J, York M, Baker J, Cottney JE, Houghton AK, Westwood PJ, Walker G (2010) Design, synthesis, and structure–activity relationships of indole-3-carboxamides as novel water soluble cannabinoid CB1 receptor agonists. Med Chem Commun 1:54

Adamowicz P (2016) Fatal intoxication with synthetic cannabinoid MDMB-CHMICA. Forensic Sci Int 261:e5–e10

Adamowicz P, Zuba D, Sekula K (2013) Analysis of UR-144 and its pyrolysis product in blood and their metabolites in urine. Forensic Sci Int 233:320–327

Adams IB, Martin BR (1996) Cannabis: pharmacology and toxicology in animals and humans. Addiction 91:1585–1614

Adams AJ, Banister SD, Irizarry L, Trecki J, Schwartz M, Gerona R (2017) "Zombie" outbreak caused by the synthetic cannabinoid AMB-FUBINACA in New York. N Engl J Med 376:235–242

Adam-Worrall J, Morrison AJ, Wishart G, Kiyoi T, Mcarthur DR (2005) Preparation of (indol-3-yl)-heterocycle derivatives as agonists of the cannabinoid CB1 receptor. World Patent 2,005,089,754

Andernach L, Pusch S, Weber C, Schollmeyer D, Munster-Muller S, Putz M, Opatz T (2016) Absolute configuration of the synthetic cannabinoid MDMB-CHMICA with its chemical characteristics in illegal products. Forensic Toxicol 34:344–352

Angerer V, Jacobi S, Franz F, Auwarter V, Pietsch J (2017) Three fatalities associated with the synthetic cannabinoids 5F-ADB, 5F-PB-22, and AB-CHMINACA. Forensic Sci Int 281:e9–e15

Angerer V, Mogler L, Steitz JP, Bisel P, Hess C, Schoeder CT, Muller CE, Huppertz LM, Westphal F, Schaper J, Auwarter V (2018) Structural characterization and pharmacological evaluation of the new synthetic cannabinoid CUMYL-PEGACLONE. Drug Test Anal 10:597–603

Babi MA, Robinson CP, Maciel CB (2017) A spicy status: synthetic cannabinoid (spice) use and new-onset refractory status epilepticus-A case report and review of the literature. SAGE Open Med Case Rep 5:2050313X17745206

Backberg M, Tworek L, Beck O, Helander A (2017) Analytically confirmed intoxications involving MDMB-CHMICA from the STRIDA project. J Med Toxicol 13:52–60

Banister SD, Wilkinson SM, Longworth M, Stuart J, Apetz N, English K, Brooker L, Goebel C, Hibbs DE, Glass M, Connor M, Mcgregor IS, Kassiou M (2013) The synthesis and pharmacological evaluation of adamantane-derived indoles: cannabimimetic drugs of abuse. ACS Chem Neurosci 4:1081–1092

Banister SD, Moir M, Stuart J, Kevin RC, Wood KE, Longworth M, Wilkinson SM, Beinat C, Buchanan AS, Glass M, Connor M, Mcgregor IS, Kassiou M (2015a) Pharmacology of indole and indazole synthetic cannabinoid designer drugs AB-FUBINACA, ADB-FUBINACA, AB-PINACA, ADB-PINACA, 5F-AB-PINACA, 5F-ADB-PINACA, ADBICA, and 5F-ADBICA. ACS Chem Neurosci 6:1546–1559

Banister SD, Stuart J, Kevin RC, Edington A, Longworth M, Wilkinson SM, Beinat C, Buchanan AS, Hibbs DE, Glass M, Connor M, Mcgregor IS, Kassiou M (2015b) Effects of bioisosteric fluorine in synthetic cannabinoid designer drugs JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135. ACS Chem Neurosci 6:1445–1458

Banister SD, Longworth M, Kevin R, Sachdev S, Santiago M, Stuart J, Mack JB, Glass M, Mcgregor IS, Connor M, Kassiou M (2016) Pharmacology of valinate and tert-leucinate synthetic cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and their analogues. ACS Chem Neurosci 7:1241–1254

Behonick G, Shanks KG, Firchau DJ, Mathur G, Lynch CF, Nashelsky M, Jaskierny DJ, Meroueh C (2014) Four postmortem case reports with quantitative detection of the synthetic cannabinoid, 5F-PB-22. J Anal Toxicol 38:559–562

Bell S, Nida C (2015) Pyrolysis of drugs of abuse: a comprehensive review. Drug Test Anal 7:445–456

Blaazer AR, Lange JH, Van Der Neut MA, Mulder A, Den Boon FS, Werkman TR, Kruse CG, Wadman WJ (2011) Novel indole and azaindole (pyrrolopyridine) cannabinoid (CB) receptor agonists: design, synthesis, structure-activity relationships, physicochemical properties and biological activity. Eur J Med Chem 46:5086–5098

Blakey K, Boyd S, Atkinson S, Wolf J, Slottje PM, Goodchild K, Mcgowan J (2016) Identification of the novel synthetic cannabimimetic 8-quinolinyl 4-methyl-3-(1-piperidinylsulfonyl)benzoate (QMPSB) and other designer drugs in herbal incense. Forensic Sci Int 260:40–53

Böhm H-J, Flohr A, Stahl M (2004) Scaffold hopping. Drug Discov Today Technol 1:217–224

Bovens M, Bissig C, Staeheli SN, Poetzsch M, Pfeiffer B, Kraemer T (2017) Structural characterization of the new synthetic cannabinoids CUMYL-PINACA, 5F-CUMYL-PINACA, CUMYL-4CN-BINACA, 5F-CUMYL-P7AICA and CUMYL-4CN-B7AICA. Forensic Sci Int 281:98–105

Bowden MJ, Williamson JPB (2014) Preparation of cannabinoid indole and indazole compounds for treating pain and nausea, stimulating appetite, and inducing a positive mood change. World Patent 2,014,167,530

Brents LK, Reichard EE, Zimmerman SM, Moran JH, Fantegrossi WE, Prather PL (2011) Phase I hydroxylated metabolites of the K2 synthetic cannabinoid JWH-018 retain *in vitro* and *in vivo* cannabinoid 1 receptor affinity and activity. PLoS One 6:e21917

Buchler IP, Hayes MJ, Hedge SG, Hockerman SL, Jones DE, Kortum SW, Rico JG, Tenbrink RE, Wu KK (2009a) Indazole derivatives as CB1 receptor modulators and their preparation and use in the treatment of CB1-mediated diseases. World Patent 2,009,106,982

Buchler IP, Hayes MJ, Hegde SG, Hockerman SL, Jones DE, Kortum SW, Rico JG, Tenbrink RE, Wu KK (2009b) Indazole derivatives as CB1 receptor modulators and their preparation and use in treatment of diseases. World Patent 2,009,106,980

Buser GL, Gerona RR, Horowitz BZ, Vian KP, Troxell ML, Hendrickson RG, Houghton DC, Rozansky D, Su SW, Leman RF (2014) Acute kidney injury associated with smoking synthetic cannabinoid. Clin Toxicol 52:664–673

Canazza I, Ossato A, Trapella C, Fantinati A, De Luca MA, Margiani G, Vincenzi F, Rimondo C, Di Rosa F, Gregori A, Varani K, Borea PA, Serpelloni G, Marti M (2016) Effect of the novel synthetic cannabinoids AKB48 and 5F-AKB48 on "tetrad", sensorimotor, neurological and neurochemical responses in mice. In vitro and in vivo pharmacological studies. Psychopharmacology 233:3685–3709

Castaneto MS, Gorelick DA, Desrosiers NA, Hartman RL, Pirard S, Huestis MA (2014) Synthetic cannabinoids: epidemiology, pharmacodynamics, and clinical implications. Drug Alcohol Depend 144:12–41

Centers for Disease Control and Prevention (2013) Acute kidney injury associated with synthetic cannabinoid use--multiple states, 2012. MMWR Morb Mortal Wkly Rep 62:93–98

Chen BC, Chen P, Hynes J, Kiener P, Leftheris K, Longphre M, Norris DJ, Pandit CR, Spergel S, Tokarski J (2002) Cannabinoid receptor modulators, their processes of preparation, and use of cannabinoid receptor modulators in treating respiratory and non-respiratory diseases. World Patent 2,001,058,869

Chimalakonda KC, Seely KA, Bratton SM, Brents LK, Moran CL, Endres GW, James LP, Hollenberg PF, Prather PL, Radominska-Pandya A, Moran JH (2012) Cytochrome P450-mediated oxidative metabolism of abused synthetic cannabinoids found in K2/Spice: identification of novel cannabinoid receptor ligands. Drug Metab Dispos 40:2174–2184

Choi H, Heo S, Kim E, Hwang BY, Lee C, Lee J (2013) Identification of (1-pentylindol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone and its 5-pentyl fluorinated analog in herbal incense seized for drug trafficking. Forensic Toxicol 31:86–92

Chung H, Choi H, Heo S, Kim E, Lee J (2014) Synthetic cannabinoids abused in South Korea: drug identifications by the National Forensic Service from 2009 to June 2013. Forensic Toxicol 32:82–88

Cohen K, Weinstein A (2018) The effects of cannabinoids on executive functions: evidence from cannabis and synthetic cannabinoids – a systematic review. Brain Sci 8:40

D'ambra TE, Eissenstat MA, Abt J, Ackerman JH, Bacon ER, Bell MR, Carabateas PM, Josef KA, Kumar V, Weaver JD, Arnold R, Casiano FM, Chippari SM, Haycock DA, Kuster JE, Luttinger DA, Stevenson JI, Ward SJ, Hill WA, Khanolkar A, Makriyannis A (1996) C-attached aminoalkylindoles: potent cannabinoid mimetics. Bioorg Med Chem Lett 6:17–22

Diao X, Scheidweiler KB, Wohlfarth A, Pang S, Kronstrand R, Huestis MA (2016a) In vitro and in vivo human metabolism of synthetic cannabinoids FDU-PB-22 and FUB-PB-22. AAPS J 18:455–464

Diao X, Scheidweiler KB, Wohlfarth A, Zhu M, Pang S, Huestis MA (2016b) Strategies to distinguish new synthetic cannabinoid FUBIMINA (BIM-2201) intake from its isomer THJ-2201: metabolism of FUBIMINA in human hepatocytes. Forensic Toxicol 34:256–267

Diao X, Wohlfarth A, Pang S, Scheidweiler KB, Huestis MA (2016c) High-resolution mass spectrometry for characterizing the metabolism of synthetic cannabinoid THJ-018 and its 5-fluoro analog THJ-2201 after incubation in human hepatocytes. Clin Chem 62:157–169

Diao X, Carlier J, Zhu M, Pang S, Kronstrand R, Scheidweiler KB, Huestis MA (2017) In vitro and in vivo human metabolism of a new synthetic cannabinoid NM-2201 (CBL-2201). Forensic Toxicol 35:20–32

Dobaja M, Grenc D, Kozelj G, Brvar M (2017) Occupational transdermal poisoning with synthetic cannabinoid cumyl-PINACA. Clin Toxicol 55:193–195

Doi T, Asada A, Takeda A, Tagami T, Katagi M, Kamata H, Sawabe Y (2016) Enantioseparation of the carboxamide-type synthetic cannabinoids N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide and methyl [1-(5-fluoropentyl)-1H-indazole-3-carbonyl]-valinate in illicit herbal products. J Chromatogr A 1473:83–89

Doi T, Tagami T, Takeda A, Asada A, Sawabe Y (2018) Evaluation of carboxamide-type synthetic cannabinoids as CB1/CB2 receptor agonists: difference between the enantiomers. Forensic Toxicol 36:51–60

European Monitoring Centre for Drugs and Drug Addiction (2014) EMCDDA–Europol 2013 annual report on the implementation of Council Decision 2005/387/JHA, implementation reports. Publications Office of the European Union, Luxembourg

European Monitoring Centre for Drugs and Drug Addiction (2016) EMCDDA–Europol 2015 annual report on the implementation of Council Decision 2005/387/JHA, implementation reports. Publications Office of the European Union, Luxembourg

European Monitoring Centre for Drugs and Drug Addiction (2017) EMCDDA–Europol 2016 annual report on the implementation of Council Decision 2005/387/JHA, implementation reports. Publications Office of the European Union, Luxembourg

Fantegrossi WE, Wilson CD, Berquist MD (2018) Pro-psychotic effects of synthetic cannabinoids: interactions with central dopamine, serotonin, and glutamate systems. Drug Metab Rev 50:65–73

Frost JM, Dart MJ, Tietje KR, Garrison TR, Grayson GK, Daza AV, El-Kouhen OF, Miller LN, Li L, Yao BB, Hsieh GC, Pai M, Zhu CZ, Chandran P, Meyer MD (2008) Indol-3-yl-tetramethylcyclopropyl ketones: effects of indole ring substitution on CB2 cannabinoid receptor activity. J Med Chem 51:1904–1912

Frost JM, Dart MJ, Tietje KR, Garrison TR, Grayson GK, Daza AV, El-Kouhen OF, Yao BB, Hsieh GC, Pai M, Zhu CZ, Chandran P, Meyer MD (2010) Indol-3-ylcycloalkyl ketones: effects of N1 substituted indole side chain variations on CB(2) cannabinoid receptor activity. J Med Chem 53:295–315

Gamage TF, Farquhar CE, Lefever TW, Marusich JA, Kevin RC, Mcgregor IS, Wiley JL, Thomas BF (2018) Molecular and behavioral pharmacological characterization of abused synthetic cannabinoids MMB- and MDMB-FUBINACA, MN-18, NNEI, CUMYL-PICA, and 5-fluoro-CUMYL-PICA. J Pharmacol Exp Ther. https://doi.org/10.1124/jpet.117.246983

Gatch MB, Forster MJ (2014) Delta9-tetrahydrocannabinol-like discriminative stimulus effects of compounds commonly found in K2/Spice. Behav Pharmacol 25:750–757
Gatch MB, Forster MJ (2015) Delta9-tetrahydrocannabinol-like effects of novel synthetic cannabinoids found on the gray market. Behav Pharmacol 26:460–468
Gatch MB, Forster MJ (2016) Delta(9)-tetrahydrocannabinol-like effects of novel synthetic cannabinoids in mice and rats. Psychopharmacology 233:1901–1910
Gatch MB, Forster MJ (2018) Delta(9)-tetrahydrocannabinol-like discriminative stimulus effects of five novel synthetic cannabinoids in rats. Psychopharmacology 235:673–680
Girreser U, Rosner P, Vasilev A (2016) Structure elucidation of the designer drug N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-3-(4-fluorophenyl)-py razole-5-carboxamide and the relevance of predicted (13) C NMR shifts – a case study. Drug Test Anal 8:668–675
Grigoryev A, Kavanagh P, Melnik A, Savchuk S, Simonov A (2013) Gas and liquid chromatography-mass spectrometry detection of the urinary metabolites of UR-144 and its major pyrolysis product. J Anal Toxicol 37:265–276
Gugelmann H, Gerona R, Li C, Tsutaoka B, Olson KR, Lung D (2014) 'Crazy Monkey' poisons man and dog: human and canine seizures due to PB-22, a novel synthetic cannabinoid. Clin Toxicol 52:635–638
Hartung B, Kauferstein S, Ritz-Timme S, Daldrup T (2014) Sudden unexpected death under acute influence of cannabis. Forensic Sci Int 237:e11–e13
Hess C, Schoeder CT, Pillaiyar T, Madea B, Muller CE (2016) Pharmacological evaluation of synthetic cannabinoids identified as constituents of spice. Forensic Toxicol 34:329–343
Hwang J, Hwang J, Ganganna B, Song I, Heo MY, Ahn S-H, Lee J (2017) Metabolic and pharmacokinetic characterization of a new synthetic cannabinoid APINAC in rats. Forensic Toxicol 36:88–101
Hynes J Jr, Leftheris K, Wu H, Pandit C, Chen P, Norris DJ, Chen B-C, Zhao R, Kiener PA, Chen X, Turk LA, Patil-Koota V, Gillooly KM, Shuster DJ, Mcintyre KW (2002) C-3 amido-indole cannabinoid receptor modulators. Bioorg Med Chem Lett 12:2399–2402
Ichikawa Y, Nakajima JI, Takahashi M, Uemura N, Yoshida M, Suzuki A, Suzuki J, Nakae D, Moriyasu T, Hosaka M (2016) Identification of (1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone (DP-UR-144) in a herbal drug product that was commercially available in the Tokyo metropolitan area. Forensic Toxicol 35:146–152
Jia W, Meng X, Qian Z, Hua Z, Li T, Liu C (2017) Identification of three cannabimimetic indazole and pyrazole derivatives, APINACA 2H-indazole analogue, AMPPPCA, and 5F-AMPPPCA. Drug Test Anal 9:248–255
Jung ME, Piizzi G (2005) gem-disubstituent effect: theoretical basis and synthetic applications. Chem Rev 105:1735–1766
Kaizaki-Mitsumoto A, Hataoka K, Funada M, Odanaka Y, Kumamoto H, Numazawa S (2017) Pyrolysis of UR-144, a synthetic cannabinoid, augments an affinity to human CB1 receptor and cannabimimetic effects in mice. J Toxicol Sci 42:335–341
Kavanagh P, Grigoryev A, Savchuk S, Mikhura I, Formanovsky A (2013) UR-144 in products sold via the Internet: identification of related compounds and characterization of pyrolysis products. Drug Test Anal 5:683–692
Kevin RC, Wood KE, Stuart J, Mitchell AJ, Moir M, Banister SD, Kassiou M, Mcgregor IS (2017) Acute and residual effects in adolescent rats resulting from exposure to the novel synthetic cannabinoids AB-PINACA and AB-FUBINACA. J Psychopharmacol 31:757–769
Kneisel S, Bisel P, Brecht V, Broecker S, Müller M, Auwärter V (2012) Identification of the cannabimimetic AM-1220 and its azepane isomer (N-methylazepan-3-yl)-3-(1-naphthoyl) indole in a research chemical and several herbal mixtures. Forensic Toxicol 30:126–134
Kondrasenko AA, Goncharov EV, Dugaev KP, Rubaylo AI (2015) CBL-2201. Report on a new designer drug: napht-1-yl 1-(5-fluoropentyl)-1H-indole-3-carboxylate. Forensic Sci Int 257:209–213
Lambeng N, Lebon F, Christophe B, Burton M, De Ryck M, Quéré L (2007) Arylsulfonamides as a new class of cannabinoid CB1 receptor ligands: identification of a lead and initial SAR studies. Bioorg Med Chem Lett 17:272–277

Lamy FR, Daniulaityte R, Nahhas RW, Barratt MJ, Smith AG, Sheth A, Martins SS, Boyer EW, Carlson RG (2017) Increases in synthetic cannabinoids-related harms: results from a longitudinal web-based content analysis. Int J Drug Policy 44:121–129

Lange JH, Attali A, van der Neut MA, Wals HC, Mulder A, Zilaout H, Duursma A, van Aken HH, van Vliet BJ (2010) Two distinct classes of novel pyrazolinecarboxamides as potent cannabinoid CB1 receptor agonists. Bioorg Med Chem Lett 20:4992–4998

Langer N, Lindigkeit R, Schiebel HM, Ernst L, Beuerle T (2014) Identification and quantification of synthetic cannabinoids in 'spice-like' herbal mixtures: a snapshot of the German situation in the autumn of 2012. Drug Test Anal 6:59–71

Langer N, Lindigkeit R, Schiebel HM, Papke U, Ernst L, Beuerle T (2016) Identification and quantification of synthetic cannabinoids in "spice-like" herbal mixtures: update of the German situation for the spring of 2016. Forensic Sci Int 269:31–41

Lee JH, Park HN, Leem T-S, Jeon J-H, Cho S, Lee J, Baek SY (2016) Identification of new synthetic cannabinoid analogue APINAC (adamantan-1-yl 1-pentyl-1H-indazole-3-carboxylate) with other synthetic cannabinoid MDMB(N)-Bz-F in illegal products. Forensic Toxicol 35:45–55

Liu C, Jia W, Hua Z, Qian Z (2017) Identification and analytical characterization of six synthetic cannabinoids NNL-3, 5F-NPB-22-7N, 5F-AKB-48-7N, 5F-EDMB-PINACA, EMB-FUBINACA, and EG-018. Drug Test Anal 9:1251–1261

Longworth M, Banister SD, Mack JB, Glass M, Connor M, Kassiou M (2016) The 2-alkyl-2H-indazole regioisomers of synthetic cannabinoids AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA are possible manufacturing impurities with cannabimimetic activities. Forensic Toxicol 34:286–303

Longworth M, Banister SD, Boyd R, Kevin RC, Connor M, Mcgregor IS, Kassiou M (2017a) Pharmacology of cumyl-carboxamide synthetic cannabinoid new psychoactive substances (NPS) CUMYL-BICA, CUMYL-PICA, CUMYL-5F-PICA, CUMYL-5F-PINACA, and their analogues. ACS Chem Neurosci 8:2159–2167

Longworth M, Connor M, Banister SD, Kassiou M (2017b) Synthesis and pharmacological profiling of the metabolites of synthetic cannabinoid drugs APICA, STS-135, ADB-PINACA, and 5F-ADB-PINACA. ACS Chem Neurosci 8:1673–1680

Louis A, Peterson BL, Couper FJ (2014) XLR-11 and UR-144 in Washington state and state of Alaska driving cases. J Anal Toxicol 38:563–568

Maeda H, Nagashima E, Hayashi YK, Kikura-Hanajiri R, Yoshida K-I (2018) MDMB-CHMICA induces thrashing behavior, bradycardia, and slow pressor response in a CB1- and CB2-receptor-dependent manner in conscious rats. Forensic Toxicol. https://doi.org/10.1007/s11419-018-0405-1

Makriyannis A, Liu Q (2003) Heteroindanes: a new class of potent cannabimimetic ligands. World Patent 2,003,035,005

Mclaughlin G, Morris N, Kavanagh PV, Power JD, Twamley B, O'brien J, Talbot B, Dowling G, Brandt SD (2016) The synthesis and characterization of the 'research chemical' N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1H-p yrazole-5-carboxamide (3,5-AB-CHMFUPPYCA) and differentiation from its 5,3-regioisomer. Drug Test Anal 8:920–929

Moloney GP, Robertson AD (2002) 3-Oxadiazol-5-yl-1-aminoalkyl-1H-indole derivatives. World Patent 2,002,036,590

Moloney GP, Angus JA, Robertson AD, Stoermer MJ, Robinson M, Wright CE, Mcrae K, Christopoulos A (2008) Synthesis and cannabinoid activity of 1-substituted-indole-3-oxadiazole derivatives: novel agonists for the CB1 receptor. Eur J Med Chem 43:513–539

Monte AA, Bronstein AC, Cao DJ, Heard KJ, Hoppe JA, Hoyte CO, Iwanicki JL, Lavonas EJ (2014) An outbreak of exposure to a novel synthetic cannabinoid. N Engl J Med 370:389–390

Morrison AJ, Adam JM, Baker JA, Campbell RA, Clark JK, Cottney JE, Deehan M, Easson A-M, Fields R, Francis S, Jeremiah F, Keddie N, Kiyoi T, Mcarthur DR, Meyer K, Ratcliffe PD, Schulz J, Wishart G, Yoshiizumi K (2011) Design, synthesis, and structure–activity relationships of indole-3-heterocycles as agonists of the CB1 receptor. Bioorg Med Chem Lett 21:506–509

Nacca N, Vatti D, Sullivan R, Sud P, Su M, Marraffa J (2013) The synthetic cannabinoid withdrawal syndrome. J Addict Med 7:296–298

Nakajima JI, Takahashi M, Seto T, Kanai C, Suzuki J, Yoshida M, Uemura N, Hamano T (2013) Analysis of azepane isomers of AM-2233 and AM-1220, and detection of an inhibitor of fatty acid amide hydrolase [3′-(aminocarbonyl)(1,1′-biphenyl)-3-yl]-cyclohexylcarbamate (URB597) obtained as designer drugs in the Tokyo area. Forensic Toxicol 31:76–85

Nakajima JI, Takahashi M, Uemura N, Seto T, Fukaya H, Suzuki J, Yoshida M, Kusano M, Nakayama H, Zaitsu K, Ishii A, Moriyasu T, Nakae D (2014) Identification of N,N-bis (1-pentylindol-3-yl-carboxy)naphthylamine (BiPICANA) found in an herbal blend product in the Tokyo metropolitan area and its cannabimimetic effects evaluated by in vitro [35S]GTPγS binding assays. Forensic Toxicol 33:84–92

Pagé D, Balaux E, Boisvert L, Liu Z, Milburn C, Tremblay M, Wei Z, Woo S, Luo X, Cheng Y-X, Yang H, Srivastava S, Zhou F, Brown W, Tomaszewski M, Walpole C, Hodzic L, St-Onge S, Godbout C, Salois D, Payza K (2008) Novel benzimidazole derivatives as selective CB2 agonists. Bioorg Med Chem Lett 18:3695–3700

Patani GA, Lavoie EJ (1996) Bioisosterism: a rational approach in drug design. Chem Rev 96:3147–3176

Pertwee RG (2005) Pharmacological actions of cannabinoids. Handb Exp Pharmacol 168:1–51

Petrov RR, Knight L, Chen SR, Wager-Miller J, Mcdaniel SW, Diaz F, Barth F, Pan HL, Mackie K, Cavasotto CN, Diaz P (2013) Mastering tricyclic ring systems for desirable functional cannabinoid activity. Eur J Med Chem 69:881–907

Rajasekaran M, Brents LK, Franks LN, Moran JH, Prather PL (2013) Human metabolites of synthetic cannabinoids JWH-018 and JWH-073 bind with high affinity and act as potent agonists at cannabinoid type-2 receptors. Toxicol Appl Pharmacol 269:100–108

Ratcliffe P, Adam JM, Baker J, Bursi R, Campbell R, Clark JK, Cottney JE, Deehan M, Easson A-M, Ecker D, Edwards D, Epemolu O, Evans L, Fields R, Francis S, Harradine P, Jeremiah F, Kiyoi T, Mcarthur D, Morrison A, Passier P, Pick J, Schnabel PG, Schulz J, Steinbrede H, Walker G, Westwood P, Wishart G, Haes JUD (2011) Design, synthesis and structure–activity relationships of (indo-3-yl) heterocyclic derivatives as agonists of the CB1 receptor. Discovery of a clinical candidate. Bioorg Med Chem Lett 21:2541–2546

Savchuk S, Appolonova S, Pechnikov A, Rizvanova L, Shestakova K, Tagliaro F (2017) In vivo metabolism of the new synthetic cannabinoid APINAC in rats by GC–MS and LC–QTOF-MS. Forensic Toxicol 35:359–368

Schep LJ, Slaughter RJ, Hudson S, Place R, Watts M (2015) Delayed seizure-like activity following analytically confirmed use of previously unreported synthetic cannabinoid analogues. Hum Exp Toxicol 34:557–560

Schindler CW, Gramling BR, Justinova Z, Thorndike EB, Baumann MH (2017) Synthetic cannabinoids found in "spice" products alter body temperature and cardiovascular parameters in conscious male rats. Drug Alcohol Depend 179:387–394

Schreiber S, Bader M, Lenchinski T, Meningher I, Rubovitch V, Katz Y, Cohen E, Gabet Y, Rotenberg M, Wolf EU, Pick CG (2018) Functional effects of synthetic cannabinoids versus delta(9)-THC in mice on body temperature, nociceptive threshold, anxiety, cognition, locomotor/exploratory parameters and depression. Addict Biol. https://doi.org/10.1111/adb.12606

Shanks KG, Behonick GS, Dahn T, Terrell A (2013) Identification of novel third-generation synthetic cannabinoids in products by ultra-performance liquid chromatography and time-of-flight mass spectrometry. J Anal Toxicol 37:517–525

Shanks KG, Winston D, Heidingsfelder J, Behonick G (2015) Case reports of synthetic cannabinoid XLR-11 associated fatalities. Forensic Sci Int 252:e6–e9

Shanks KG, Clark W, Behonick G (2016) Death associated with the use of the synthetic cannabinoid ADB-FUBINACA. J Anal Toxicol 40:236–239

Shevyrin V, Melkozerov V, Nevero A, Eltsov O, Morzherin Y, Shafran Y (2013a) Identification and analytical properties of new synthetic cannabimimetics bearing 2,2,3,3-tetramethylcyclopropanecarbonyl moiety. Forensic Sci Int 226:62–73

Shevyrin V, Melkozerov V, Nevero A, Eltsov O, Shafran Y (2013b) Analytical characterization of some synthetic cannabinoids, derivatives of indole-3-carboxylic acid. Forensic Sci Int 232:1–10

Shevyrin V, Melkozerov V, Nevero A, Eltsov O, Morzherin Y, Shafran Y (2014) 3-Naphthoylindazoles and 2-naphthoylbenzoimidazoles as novel chemical groups of synthetic cannabinoids: chemical structure elucidation, analytical characteristics and identification of the first representatives in smoke mixtures. Forensic Sci Int 242:72–80

Shevyrin V, Melkozerov V, Eltsov O, Shafran Y, Morzherin Y (2016a) Synthetic cannabinoid 3-benzyl-5-[1-(2-pyrrolidin-1-ylethyl)-1H-indol-3-yl]-1,2,4-oxadiazole. The first detection in illicit market of new psychoactive substances. Forensic Sci Int 259:95–100

Shevyrin V, Melkozerov V, Endres GW, Shafran Y, Morzherin Y (2016b) On a new cannabinoid classification system: a sight on the illegal market of novel psychoactive substances. Cannabis Cannabinoid Res 1:186–194

Silva JP, Carmo H, Carvalho F (2018) The synthetic cannabinoid XLR-11 induces in vitro nephrotoxicity by impairment of endocannabinoid-mediated regulation of mitochondrial function homeostasis and triggering of apoptosis. Toxicol Lett 287:59–69

Simolka K, Lindigkeit R, Schiebel H-M, Papke U, Ernst L, Beuerle T (2012) Analysis of synthetic cannabinoids in "spice-like" herbal highs: snapshot of the German market in summer 2011. Anal Bioanal Chem 404:157–171

Sobolevsky T, Prasolov I, Rodchenkov G (2012) Detection of urinary metabolites of AM-2201 and UR-144, two novel synthetic cannabinoids. Drug Test Anal 4:745–753

Springer YP, Gerona R, Scheunemann E, Shafer SL, Lin T, Banister SD, Cooper MP, Castrodale LJ, Levy M, Butler JC, Mclaughlin JB (2016) Increase in adverse reactions associated with use of synthetic cannabinoids – Anchorage, Alaska, 2015-2016. MMWR Morb Mortal Wkly Rep 65:1108–1111

Surmont T, Daníelsson HV, Hughes B, Sedefov R (2017) The "Spice" trade. Int Crim Just Rev: 105756771774534

Tai S, Fantegrossi WE (2017) Pharmacological and toxicologicaleffects of synthetic cannabinoids and their metabolites. In: Baumann MH, Glennon RA, Wiley JL (eds) Neuropharmacology of new psychoactive substances (NPS): the science behind the headlines. Springer, Cham

Tait RJ, Caldicott D, Mountain D, Hill SL, Lenton S (2016) A systematic review of adverse events arising from the use of synthetic cannabinoids and their associated treatment. Clin Toxicol 54:1–13

Talele TT (2018) Natural-products-inspired use of the gem-dimethyl group in medicinal chemistry. J Med Chem 61:2166–2210

Tapp L, Ramsey JG, Wen A, Gerona R (2017) Synthetic cannabinoid and mitragynine exposure of law enforcement agents during the raid of an illegal laboratory – Nevada, 2014. MMWR Morb Mortal Wkly Rep 66:1291–1294

Theunissen EL, Hutten N, Mason NL, Toennes SW, Kuypers KPC, De Sousa Fernandes Perna EB, Ramaekers JG (2018) Neurocognition and subjective experience following acute doses of the synthetic cannabinoid JWH-018: a phase 1, placebo-controlled, pilot study. Br J Pharmacol 175:18–28

Thomas BF, Lefever TW, Cortes RA, Grabenauer M, Kovach AL, Cox AO, Patel PR, Pollard GT, Marusich JA, Kevin RC, Gamage TF, Wiley JL (2017) Thermolytic degradation of synthetic cannabinoids: chemical exposures and pharmacological consequences. J Pharmacol Exp Ther 361:162–171

Thornton SL, Wood C, Friesen MW, Gerona RR (2013) Synthetic cannabinoid use associated with acute kidney injury. Clin Toxicol 51:189–190

Trecki J, Gerona RR, Schwartz MD (2015) Synthetic cannabinoid-related illnesses and deaths. N Engl J Med 373:103–107

Tsujikawa K, Yamamuro T, Kuwayama K, Kanamori T, Iwata YT, Inoue H (2013) Thermal degradation of a new synthetic cannabinoid QUPIC during analysis by gas chromatography–mass spectrometry. Forensic Toxicol 32:201–207

Tyndall JA, Gerona R, De Portu G, Trecki J, Elie MC, Lucas J, Slish J, Rand K, Bazydlo L, Holder M, Ryan MF, Myers P, Iovine N, Plourde M, Weeks E, Hanley JR, Endres G, St Germaine D, Dobrowolski PJ, Schwartz M (2015) An outbreak of acute delirium from exposure to the synthetic cannabinoid AB-CHMINACA. Clin Toxicol 53:950–956

Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y (2012) Identification of two new-type synthetic cannabinoids, N-(1-adamantyl)-1-pentyl-1*H*-indole-3-carboxamide (APICA) and N-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide (APINACA), and detection of five synthetic cannabinoids, AM-1220, AM-2233, AM-1241, CB-13 (CRA-13), and AM-1248, as designer drugs in illegal products. Forensic Toxicol 30:114–125

Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y (2013a) URB-754: a new class of designer drug and 12 synthetic cannabinoids detected in illegal products. Forensic Sci Int 227:21–32

Uchiyama N, Matsuda S, Kawamura M, Kikura-Hanajiri R, Goda Y (2013b) Two new-type cannabimimetic quinolinyl carboxylates, QUPIC and QUCHIC, two new cannabimimetic carboxamide derivatives, ADB-FUBINACA and ADBICA, and five synthetic cannabinoids detected with a thiophene derivative α-PVT and an opioid receptor agonist AH-7921 identified in illegal products. Forensic Toxicol 31:223–240

Uchiyama N, Matsuda S, Kawamura M, Kikura-Hanajiri R, Goda Y (2014a) Identification of two new-type designer drugs, piperazine derivative MT-45 (I-C6) and synthetic peptide Noopept (GVS-111), with synthetic cannabinoid A-834735, cathinone derivative 4-methoxy-α-PVP, and phenethylamine derivative 4-methylbuphedrine from illegal products. Forensic Toxicol 32:9–18

Uchiyama N, Matsuda S, Kawamura M, Shimokawa Y, Kikura-Hanajiri R, Aritake K, Urade Y, Goda Y (2014b) Characterization of four new designer drugs, 5-chloro-NNEI, NNEI indazole analog, alpha-PHPP and alpha-POP, with 11 newly distributed designer drugs in illegal products. Forensic Sci Int 243:1–13

Uchiyama N, Shimokawa Y, Kawamura M, Kikura-Hanajiri R, Hakamatsuka T (2014c) Chemical analysis of a benzofuran derivative, 2-(2-ethylaminopropyl)benzofuran (2-EAPB), eight synthetic cannabinoids, five cathinone derivatives, and five other designer drugs newly detected in illegal products. Forensic Toxicol 32:266–281

Uchiyama N, Shimokawa Y, Matsuda S, Kawamura M, Kikura-Hanajiri R, Goda Y (2014d) Two new synthetic cannabinoids, AM-2201 benzimidazole analog (FUBIMINA) and (4-methylpiperazin-1-yl)(1-pentyl-1H-indol-3-yl)methanone (MEPIRAPIM), and three phenethylamine derivatives, 25H-NBOMe 3,4,5-trimethoxybenzyl analog, 25B-NBOMe, and 2C-N-NBOMe, identified in illegal products. Forensic Toxicol 32:105–115

Uchiyama N, Asakawa K, Kikura-Hanajiri R, Tsutsumi T, Hakamatsuka T (2015a) A new pyrazole-carboxamide type synthetic cannabinoid AB-CHFUPYCA [N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1H-pyrazole-5-carboxamide] identified in illegal products. Forensic Toxicol 33:367–373

Uchiyama N, Shimokawa Y, Kikura-Hanajiri R, Demizu Y, Goda Y, Hakamatsuka T (2015b) A synthetic cannabinoid FDU-NNEI, two 2-indazole isomers of synthetic cannabinoids AB-CHMINACA and NNEI indazole analog (MN-18), a phenethylamine derivative -OH-EDMA, and a cathinone derivative dimethoxy-alpha-PHP, newly identified in illegal products. Forensic Toxicol 33:244–259

Uemura N, Fukaya H, Kanai C, Yoshida M, Nakajima JI, Takahashi M, Suzuki J, Moriyasu T, Nakae D (2014) Identification of a synthetic cannabinoid A-836339 as a novel compound found in a product. Forensic Toxicol 32:45–50

Usui K, Fujita Y, Kamijo Y, Kokaji T, Funayama M (2018) Identification of 5-Fluoro ADB in human whole blood in four death cases. J Anal Toxicol 42:e21–e25

Van Hout MC, Hearne E (2016) User experiences of development of dependence on the synthetic cannabinoids, 5f-AKB48 and 5F-PB-22, and subsequent withdrawal syndromes. Int J Ment Heal Addict 15:565–579

Vineis P, Pirastu R (1997) Aromatic amines and cancer. Cancer Causes Control 8:346–355

Weber C, Pusch S, Schollmeyer D, Münster-Müller S, Pütz M, Opatz T (2016) Characterization of the synthetic cannabinoid MDMB-CHMCZCA. Beilstein J Org Chem 12:2808–2815

Westphal F, Sonnichsen FD, Knecht S, Auwarter V, Huppertz L (2015) Two thiazolylindoles and a benzimidazole: novel compounds on the designer drug market with potential cannabinoid receptor activity. Forensic Sci Int 249:133–147

Wiley JL, Marusich JA, Lefever TW, Grabenauer M, Moore KN, Thomas BF (2013) Cannabinoids in disguise: delta9-tetrahydrocannabinol-like effects of tetramethylcyclopropyl ketone indoles. Neuropharmacology 75:145–154

Wiley JL, Marusich JA, Lefever TW, Antonazzo KR, Wallgren MT, Cortes RA, Patel PR, Grabenauer M, Moore KN, Thomas BF (2015) AB-CHMINACA, AB-PINACA, and FUBIMINA: affinity and potency of novel synthetic cannabinoids in producing delta9-tetrahydrocannabinol-like effects in mice. J Pharmacol Exp Ther 354:328–339

Wiley JL, Lefever TW, Marusich JA, Grabenauer M, Moore KN, Huffman JW, Thomas BF (2016) Evaluation of first generation synthetic cannabinoids on binding at non-cannabinoid receptors and in a battery of in vivo assays in mice. Neuropharmacology 110:143–153

Willis PG, Pavlova OA, Chefer SI, Vaupel DB, Mukhin AG, Horti AG (2005) Synthesis and structure-activity relationship of a novel series of aminoalkylindoles with potential for imaging the neuronal cannabinoid receptor by positron emission tomography. J Med Chem 48:5813–5822

Wohlfarth A, Gandhi AS, Pang S, Zhu M, Scheidweiler KB, Huestis MA (2014) Metabolism of synthetic cannabinoids PB-22 and its 5-fluoro analog, 5F-PB-22, by human hepatocyte incubation and high-resolution mass spectrometry. Anal Bioanal Chem 406:1763–1780

Wrobleski ST, Chen P, Hynes J, Lin S, Norris DJ, Pandit CR, Spergel S, Wu H, Tokarski JS, Chen X, Gillooly KM, Kiener PA, Mcintyre KW, Patil-Koota V, Shuster DJ, Turk LA, Yang G, Leftheris K (2003) Rational design and synthesis of an orally active indolopyridone as a novel conformationally constrained cannabinoid ligand possessing antiinflammatory properties. J Med Chem 46:2110–2116

Wurita A, Hasegawa K, Minakata K, Gonmori K, Nozawa H, Yamagishi I, Watanabe K, Suzuki O (2015) Identification and quantitation of 5-fluoro-ADB-PINACA and MAB-CHMINACA in dubious herbal products. Forensic Toxicol 33:213–220

Yao BB, Hsieh G, Daza AV, Fan Y, Grayson GK, Garrison TR, El Kouhen O, Hooker BA, Pai M, Wensink EJ, Salyers AK, Chandran P, Zhu CZ, Zhong C, Ryther K, Gallagher ME, Chin CL, Tovcimak AE, Hradil VP, Fox GB, Dart MJ, Honore P, Meyer MD (2009) Characterization of a cannabinoid CB2 receptor-selective agonist, A-836339 [2,2,3,3-tetramethyl-cyclopropanecarboxylic acid [3-(2-methoxy-ethyl)-4,5-dimethyl-3H-thiazol-(2Z)-ylidene]-amide], using in vitro pharmacological assays, in vivo pain models, and pharmacological magnetic resonance imaging. J Pharmacol Exp Ther 328:141–151

Yildirim M, Wals HC, Van VBJ, Lange JHM (2009) 4,5-dihydro-(1h)-pyrazole derivatives as cannabinoid cb1 receptor modulators. World Patent 2,008,152,086

You H, Gadotti VM, Petrov RR, Zamponi GW, Diaz P (2011) Functional characterization and analgesic effects of mixed cannabinoid receptor/T-type channel ligands. Mol Pain 7:89

Zanda MT, Fattore L (2018) Old and new synthetic cannabinoids: lessons from animal models. Drug Metab Rev 50:54–64

Zuba D, Geppert B, Sekuła K, Żaba C (2013) [1-(Tetrahydropyran-4-ylmethyl)-1H-indol-3-yl]-(2,2,3,3-tetramethylcyclopropyl)methanone: a new synthetic cannabinoid identified on the drug market. Forensic Toxicol 31:281–291

Zuurman L, Passier PC, De Kam M, Kleijn HJ, Cohen AF, Van Gerven JM (2009) Pharmacodynamic and pharmacokinetic effects of the intravenously administered CB1 receptor agonist Org 28611 in healthy male volunteers. J Psychopharmacol 23:633–644

Serotonergic Psychedelics: Experimental Approaches for Assessing Mechanisms of Action

Clinton E. Canal

Contents

Abstract

Recent, well-controlled – albeit small-scale – clinical trials show that serotonergic psychedelics, including psilocybin and lysergic acid diethylamide, possess great promise for treating psychiatric disorders, including treatment-resistant depression. Additionally, fresh results from a deluge of clinical neuroimaging studies are unveiling the dynamic effects of serotonergic psychedelics on functional activity within, and connectivity across, discrete neural systems. These observations have led to testable hypotheses regarding neural processing mechanisms that contribute to psychedelic effects and therapeutic benefits.

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Despite these advances and a plethora of preclinical and clinical observations supporting a central role for brain serotonin 5-HT_{2A} receptors in producing serotonergic psychedelic effects, lingering and new questions about mechanisms abound. These chiefly pertain to molecular neuropharmacology. This chapter is devoted to illuminating and discussing such questions in the context of preclinical experimental approaches for studying mechanisms of action of serotonergic psychedelics, classic and new.

Keywords

α-Adrenergic · 5-HT_{2A} · 5-HT_{2C} · Cingulate cortex · Head-twitch · Ketanserin · Psychedelic mechanisms · Receptor binding · Receptor conformations · Receptor dimers · Receptor function · Serotonin · Signal transduction

Abbreviations

1P-LSD	1-Propionyl-lysergic acid diethylamide
25C-NBOMe	*N*-(2-Methoxybenzyl)-2,5-dimethoxy-4-bromophenethylamine
25CN-NBOH	*N*-(2-Hydroxybenzyl)-2,5-dimethoxy-4-cyanophenylethylamine
25I-NBOMe	*N*-(2-Methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine
2C-B	4-Bromo-2,5-dimethoxyphenethylamine
2C-I	4-Iodo-2,5-dimethoxyphenethylamine
2C-T-7	2,5-Dimethoxy-4-propylthiophenethylamine
5-APB	5-(2-Aminopropyl)benzofuran
5-HT	5-Hydroxytryptamine (serotonin)
5-MeO-DALT	5-Methoxy-*N,N*-diallyltryptamine
5-MeO-DIPT	5-Methoxy-*N,N*-diisopropyltryptamine
5-MeO-DMT	5-Methoxy-*N,N*-dimethyltryptamine
6-APB	6-(2-Aminopropyl)benzofuran
AL-LAD	N^6-allyl-6-norlysergic acid diethylamide
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
bk-2C-B	β-Keto-2,5-dimethoxy-4-bromophenethylamine
BOL-148	2-Bromo-lysergic acid diethylamide
CB1	Cannabinoid 1 receptor
DA	Dopamine
DIPT	*N,N*-Diisopropyltryptamine
DMT	*N,N*-Dimethyltryptamine
DOB	2,5-Dimethoxy-4-bromoamphetamine
DOI	2,5-Dimethoxy-4-iodoamphetamine
DOM	2,5-Dimethoxy-4-methylamphetamine
DOPAC	3,4-Dihydroxyphenylacetic acid
DPT	*N,N*-Dipropyltryptamine
IP_3	Inositol 1,4,5-trisphosphate
LSA	Lysergamide
LSD	Lysergic acid diethylamide

LSM-775	Lysergic acid morpholide
LSZ	Lysergic acid 2,4-dimethylazetidide
mCPP	*meta*-Chlorophenylpiperazine
MDMA	3,4-Methylenedioxymethamphetamine
mGluR2	Metabotropic glutamate receptor 2
NMDA	*N*-Methyl-D-aspartate
PARGY-LAD	N^6-Propynyl-6-norlysergic acid diethylamide
PET	Positron emission tomography
SERT	Serotonin transporter
TAAR1	Trace amine-associated receptor 1
TCB-2	1-(3-Bromo-2,5-dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl) methanamine
THC	Δ^9-Tetrahydrocannabinol
THH	Tetrahydroharmine

1 Introduction

Humans have been reporting their experiences with psychoactive substances in exquisite detail for centuries, at least. In the mid 1800s, J. J. Moreau de Tours wrote about hashish intoxication (Moreau 1973). Sigmund Freud later scripted his personal relationship with cocaine (Freud and Byck 1975). Ann and Alexander Shulgin then gave the world *PiHKAL* (Shulgin and Shulgin 1991) and *TiHKAL* (Shulgin and Shulgin 1997), books describing mostly novel psychedelic drugs and their effects. Fast-forward to today, we see the practice of self-reporting experiences with psychoactive substances is rampant, organized, archived, and accessible (e.g., Erowid.org). The surge of information about new psychoactive substances reported on the internet paralleling the surge in online vendors selling such "research chemicals" has catalyzed their spread and use. Systematic research aimed to discover and document their unique mechanisms has, understandably, lagged behind.

Fortunately for researchers, most novel psychedelics still fit within familiar chemotype classes and have overlapping pharmacology with their classic predecessors—mainly 5-$HT_{2(A,\ B,\ and\ C)}$ receptor agonist activity in the case of serotonergic psychedelics that include lysergamides (e.g., LSD), tryptamines (e.g., psilocybin and DMT), and phenethylamines (e.g., mescaline and including phenylisopropylamines, e.g., DOB). Chemists have synthesized dozens of relatively obscure serotonergic psychedelics, which have appeared recently on the clandestine market. Examples include lysergamides like AL-LAD (Brandt et al. 2017b) and PARGY-LAD, tryptamines like 5-MeO-DALT (Cozzi and Daley 2016), and phenylethylamines like bk-2C-B, the β-ketone analog of 2C-B and 25C-NBOMe, another analog of 2C-B (Halberstadt 2017). Recent literature covers what is currently known regarding the physiological, psychological, and visual perceptual effects of serotonergic psychedelics, their neuropharmacology and effects on human brain functional connectivity, their use as potential medicines, their inherent

risks, and the phenomenology of the psychedelic experience (Halberstadt 2017; Kometer and Vollenweider 2016; Liechti 2017; Nichols 2016; Nichols et al. 2017; Preller and Vollenweider 2016). This chapter covers preclinical research strategies used to elucidate common and divergent mechanisms of serotonergic psychedelics, classic and new.

2 5-HT_{2A} Receptors: The End of the Beginning

For research probing mechanisms, it is clear we have reached the end of the beginning—a cadre of researchers agree that 5-HT_{2A} receptor activation is necessary for most of the psychoactive effects of serotonergic psychedelics (Kometer et al. 2013; Kraehenmann et al. 2017; Nichols 2016; Preller et al. 2017; Vollenweider et al. 1998). To understand mechanisms, though, requires delineating atomic-level drug and receptor interactions and attendant consequences on signal transduction (Wacker et al. 2017) and cellular intrinsic excitability, subsequent short-term and long-term effects on electrochemical communication within and between micro- and macroneural circuits (Petri et al. 2014), as well as the interplay of neural circuits with the user's personality, psychological and cognitive state, personal history and genetic background ("set"), and the external environment ("setting") (Studerus et al. 2012).

Questions from these wide-ranging levels of analyses remain. At the highest level, self-reported subjective experiences, there appear to be differences in effects (beyond pharmacokinetics) across and even within classes of serotonergic psychedelics (Glennon 1992; Shulgin and Shulgin 1991, 1997). Although representatives from each chemotype class can induce similar, visual "form constants" of the lattice and tunnel types (elementary psychedelic patterns) (Kometer and Vollenweider 2016), users typically report differences in their "body load," stimulant and entactogenic effects, the degree to which they produce "organic" or "synthetic" visual or aural hallucinations, and how deeply and clearly they affect emotional and cognitive states. For example, the psychedelic 5-HT_{2A} agonist, DIPT, appears to produce distinct aural, hallucinatory effects (Blough et al. 2014; Rickli et al. 2016; Shulgin and Shulgin 1997), whereas DMT (and analogs including 5-MeO-DMT) is distinguished by its marked proclivity to induce complex hallucinations (Strassman 2001), such as visual hallucinations of things, entities, or events separated from consensus reality. If the effects of different serotonergic psychedelics can be distinguished reliably, then what, mechanistically, differentiates them? Are differences caused by unique, dynamic, active conformations of 5-HT_{2A} receptors, or do some serotonergic psychedelics preferentially target distinct populations of 5-HT_{2A} receptors, e.g., pre- or postsynaptic (Bécamel et al. 2017)? Alternatively, other receptor targets or 5-HT_{2A} receptors functionally linked to other systems, for example, the endocannabinoid system (Best and Regehr 2008; Parrish and Nichols 2006), may contribute to psychedelic effects. Furthermore, unique 5-HT_2 receptor homo- or heterodimers or oligomers could be the mechanistic target of serotonergic psychedelics.

3 Binding Events and Cellular Signal Transduction

3.1 Radioligand Receptor Binding

Radioligand receptor binding assays were integral in determining that 5-HT_2 receptors were the primary targets of classic psychedelics, such as LSD and DOB (Glennon et al. 1984), and radiolabeled psychedelics applied in autoradiography of brain of slices determined the location of psychedelic receptor targets across neural systems in rodents (McKenna et al. 1987; McKenna and Saavedra 1987). Presently, researchers are developing selective 5-HT_{2A} agonist radioligands to analyze 5-HT_{2A} receptors in humans, using PET imaging techniques (Johansen et al. 2017), and a recent clinical study employing [^{11}C]Cimbi-36, a selective 5-HT_2 agonist, reports dense expression of 5-HT_2 receptors across all cortical regions, but limited expression in subcortical regions (Beliveau et al. 2017).

Radioligand competition binding and saturation binding assays are used to quantify the affinity, or strength of interaction, of new compounds at receptors. In a competition binding assay, a compound with unknown affinity at the receptor of interest is added to a multi-well microplate at increasing concentrations across wells, typically at half-log units, together with a radiolabeled ligand, with known affinity at the receptor of interest, at one fixed concentration (e.g., its K_d at the receptor, the equilibrium dissociation constant of the ligand). Cell membranes expressing the receptor are then added to the wells, and the mixture is incubated for a period of time until equilibrium is achieved, i.e., the amount of free ligand and ligand bound to the receptor remains constant. The contents of the wells are then rapidly passed through a fiberglass filter mat that collects radioligand bound to receptor but permits free radioligand to pass through. Samples are then added to scintillation cocktail, and a scintillation counter detects the amount of radioactivity emitted from each sample over a fixed time (e.g., counts per minute). The affinity, K_i (equilibrium dissociation constant of a ligand determined in inhibition studies), of the unknown is then determined by fitting a nonlinear dose–response inhibition curve, calculating the concentration of the compound required to displace 50% of the radioligand from the receptor, followed by correction incorporating the radioligand's affinity and concentration (Cheng and Prusoff 1973).

Saturation binding assays include a similar workflow, but the radionuclide is attached chemically to the compound with unknown affinity at the receptor of interest. The radioligand is then incubated, with tissue expressing the receptor, at increasing concentrations across wells. The affinity, K_d, of the radioligand, or the concentration required to occupy 50% of the receptors at equilibrium, is then calculated, as well as the saturating concentration, which can be used to calculate the density of receptor binding sites labeled by the radioligand, or B_{max}. For details, refer to McKinney and Raddatz (2006). A saturation binding assay provides a more accurate affinity value than a single competition binding assay, due to the fact that a radioligand in a competition assay may selectively bind a subset of receptors existing in specific conformations—or may interact with unique receptor amino acids with which the unknown compound may not interact. In other words, K_i values at a specific receptor population may be different depending on the radioligand used, but K_d values remain constant.

Agonist affinities at G-protein-coupled receptors (GPCRs) are strongly affected by the state of G-proteins linked to them. Agonists stabilize active receptor conformations—receptors bound to guanine nucleotide-free Gα proteins—and then dissociate slowly from these active conformations, imparting high affinity at them (DeVree et al. 2016). Thus, competition binding assays in the presence or absence of non-hydrolyzable guanine nucleotides (e.g., GTPγS which blocks agonist high-affinity binding) can be used to determine the affinity of ligands at inactive or active receptor conformations. Moreover, since agonist ligands typically have a higher affinity at receptors labeled with agonists (high-affinity, K_H) compared to antagonists (low-affinity, K_L), comparisons of these affinities can be used as first-pass screens to test novel ligands for agonist activity. Also, functional efficacies of 5-HT_2 ligands correlate strongly with their K_L to K_H ratios (Egan et al. 2000).

Radioligand binding assays can also be used to measure ligand–receptor association and dissociation rates (Sykes et al. 2010). Ligand–receptor kinetics data can inform efficacy, selectivity, and duration of action in vivo (de Witte et al. 2016). A ligand's residence time at a receptor—the duration of time a ligand is bound to a receptor—may also be a critical factor for recruiting intracellular signaling molecules. Receptor-mediated activation of cellular cascades may be time-dependent; if an agonist ligand's dissociation rate is too quick, a substantial proportion of a cellular cascade may remain inactive. Recently, scientists showed that LSD has a relatively long residence time at the 5-HT_{2A} receptor (Wacker et al. 2017). Such studies may inform mechanistic differences between serotonergic psychedelics. The actions of DMT, for example, are very short-lived compared to LSD. It may be discovered that residence time dictates transduction signals that initiate unique cellular psychedelic cascades. LSD potently recruits β-arrestin2 to 5-HT_{2A} receptors, but mutating lysine residue 229 to alanine to decrease LSD's residence time strongly reduces β-arrestin2 recruitment to 5-HT_{2A} (Wacker et al. 2017).

Classic psychedelics of the tryptamine and lysergamide chemotypes are not selective for 5-HT_2 receptors. Psilocin, for example, has appreciable affinity (between 4 and 220 nM K_i) at human 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_5, 5-HT_6, and 5-HT_7 receptors, c.f. PDSP database (Roth et al. 2000). Novel serotonergic psychedelics, such as *N*-benzylphenethylamines, and putatively psychedelic *N*-benzylated-5-methoxytryptamines, possess high affinity at 5-HT_2 receptors but also bind to other receptors. For example, 25I-NBOMe has significant affinity (<300 nM K_i) at μ-opioid, κ-opioid, and histamine H1 receptors (Nichols et al. 2008). Others have also observed activity of certain *N*-benzylphenethylamines at H1 (K_i as low as 80 nM), α1A- and α2A-adrenergic (K_i as low as ~300 nM) receptors (Rickli et al. 2015b), rendering them somewhat similar to LSD, which is promiscuous (PDSP database). Relative to their 2C-x classic hallucinogen predecessors, these drugs have substantially greater selectivity for 5-HT_2 over 5-HT_{1A} receptors (Rickli et al. 2015b). Many *N*-benzylated-5-methoxytryptamines and *N*-benzylphenethylamines also possess significant affinity at 5-HT_6, and *N*-3-iodobenzyl-5-methoxytryptamine also has significant activity at 5-HT_7 receptors (Nichols et al. 2015). A recent report of 25CN-NBOH, however, shows that this

compound is very selective at 5-HT_2 receptors compared to a host of other receptors; the only relevant affinities (K_i ~300 nM or less) noted are at human 5-HT_6 and rat sigma-1 and sigma-2 (Jensen et al. 2017).

3.2 Receptor Functional Assays

Serotonergic psychedelics stimulate 5-HT_2 receptor – $G\alpha_q$ signaling, as measured by activation of phospholipase C-β, causing increases in phosphoinositide hydrolysis, thereby stimulating inositol phosphate production and activation of protein kinase C (Jope et al. 1994; Sanders-Bush et al. 1988); this is the canonical 5-HT_2 receptor pathway. Most experimenters focus on activation of Gα subunits, because of technical challenges measuring βγ activation from $G\alpha_q$-coupled receptors (Kadamur and Ross 2013). Laborious techniques to assess ligand functional effects involving chromatography-based detection or anion exchange columns (Felder et al. 1990; Hide et al. 1989) have frequently been replaced by relatively simple, high-throughput, kit assays that employ highly selective, fluorophore-labeled monoclonal antibodies that bind signaling molecules (Canal et al. 2013b). With the advent of protocols and toolkits to measure ligand-stimulated 5-HT_2–β-arrestin2 recruitment, researchers increasingly examine this event, especially with the objective to determine ligand bias (Kenakin 2016; Wacker et al. 2017; Wang et al. 2013).

Other signaling pathways activated by 5-HT_2 receptors, such as $G\alpha_{13}$-dependent or $G\alpha_{13}$-independent phospholipase D pathways (Barclay et al. 2011; McGrew et al. 2002), remain difficult to measure. The workflow can include extraction steps and chromatography and is low-throughput (Walker et al. 2004). Accordingly, much less is known about how psychedelics affect these cellular signaling cascades. For similar reasons, the activity of psychedelics is not fully characterized at other members of the $G\alpha_q$ subclass or other signaling pathways that may be linked endogenously to 5-HT_2 receptors, including $G\alpha_q$–p63RhoGEF-RhoA, $G\alpha_{11}$, $G\alpha_{12}$, $G\alpha_{14}$, $G\alpha_{15}$, and $G\alpha_{16}$ (Milligan and Kostenis 2006). 5-HT_2 receptor activation stimulates calcium mobilization, but this response can be triggered by $G\alpha_q$-dependent increases in inositol phosphates, i.e. IP_3, that activate IP_3 receptors (Ca^{2+} release channels) on endoplasmic reticulum, or by activation of Ca^{2+} permeable channels on cell membranes. There is much to be discovered about the ability of 5-HT_2 receptors to modulate activity of distinct ion channels important for neurotransmission. It is incumbent to determine the effects of serotonergic psychedelics at noncanonical signal transduction pathways, because the 5-HT_{2A} intracellular signals that generate psychedelic effects remain mysterious (Nichols 2016).

Although there are correlations between the psychedelic potencies of phenethylamines, their phenylisopropylamine counterparts, and 5-HT_{2A} agonist efficacy to stimulate inositol phosphate production (Moya et al. 2007; Parrish et al. 2005), LSD, one of the most potent psychedelics, is a notoriously weak 5-HT_{2A} agonist at this pathway (Berg et al. 1998; Egan et al. 1998a). Moreover, high-affinity agonist binding of several 5-HT_{2A} ligands does not correlate with efficacy to stimulate 5-HT_{2A}-mediated inositol phosphate production (Roth et al. 1997). Also, ligand

efficacy at this pathway does not correlate with efficacy to substitute for LSD or DOM in drug discrimination tests (Rabin et al. 2002). Serotonergic psychedelics are most often observed to be partial agonists at the 5-HT_{2A} inositol phosphate production pathway, relative to 5-HT. This extends across all classes of serotonergic psychedelics, classic and new, including *N*-benzylphenethylamines, DOB, 2C-B, novel tryptamines, psilocin, and LSD (Acuna-Castillo et al. 2002; Moya et al. 2007; Parrish et al. 2005; Rickli et al. 2015b, 2016).

Lorcaserin (Belviq®, a 5-HT_{2C}-preferring agonist for obesity), however, efficaciously stimulates 5-HT_{2A}-mediated inositol phosphate production in vitro (75% efficacy compared to 5-HT at human 5-HT_{2A} receptors) (Thomsen et al. 2008), and at clinical doses, likely stimulates this 5-HT_{2A} receptor pathway. From the FDA briefing document (NDA22529):

> Assuming that distribution of lorcaserin in monkeys and humans is most comparable, brain levels of lorcaserin may reach 430 ng/ml or 1.7 μM from the clinical dose of 10mg bid. This concentration of lorcaserin would be expected to activate central 5HT2A and potentially [5HT]2B receptors, assuming that lorcaserin has access to receptor sites in the CNS.

Yet, at this dose, lorcaserin is not psychedelic. As a benzazapine, it is also structurally quite different from any of the serotonergic psychedelics—rehashing thoughts that chemotype, and by extension chemotype-dependent stabilization of special 5-HT_{2A} psychedelic conformations, drives psychedelic effects. Other structurally unique ligands that activate 5-HT_{2A}–$G\alpha_q$ signaling, including the piperazine mCPP, also do not elicit psychoactive effects like the serotonergic psychedelics. It has been argued that increased activity at 5-HT_{2C} relative to 5-HT_{2A} receptors, which mCPP and lorcaserin possess, attenuates psychedelic effects (Fantegrossi et al. 2010), but several observations disprove this postulation (Canal and Morgan 2012). For example, all serotonergic psychedelics are 5-HT_{2C} agonists, and psychedelic effects do not abate by increasing dose, i.e., by increasing stimulation of 5-HT_{2C} receptors.

The classic first-generation antipsychotic, chlorpromazine (Thorazine®), a potent antagonist at the 5-HT_{2A}–$G\alpha_q$–inositol phosphate pathway (Canton et al. 1994), does "not significantly influence the somatic and psychological disturbances" caused by LSD (Clark and Bliss 1957). Others reported that chlorpromazine attenuates, but does not fully block, the psychedelic effects of LSD (Isbell and Logan 1957) or DOM (Snyder et al. 1967)—chlorpromazine doses ranged from 50 to 200 mg (P.O.). Also peculiar is that the non-hallucinogenic 5-HT_{2A} lysergamides, ergotamine and BOL-148, do not significantly alter the psychoactive effects of psilocybin or LSD, respectively, in humans (Clark and Bliss 1957; Pokorny et al. 2016). Though, ergotamine may not readily cross the blood–brain barrier (Verhoeff et al. 1993), and BOL-148 appears to elicit psychoactive effects in some people (Richards et al. 1958). Collectively, these data cast major doubt on the 5-HT_2–$G\alpha_q$–inositol phosphate pathway as a central mediator of psychedelic effects. Direct in vivo support for this conclusion emanates from a preclinical study that showed knockout of $G\alpha_q$ does not eliminate (but does attenuate) the 5-HT_{2A}-dependent head-twitch response induced by the psychedelic phenylisopropylamine DOI (Garcia et al. 2007).

Knockout of β-arrestin2 in mice has *no* effect on the DOI-elicited head-twitch response (Schmid et al. 2008) and actually increases the head-twitch response elicited by 5-MeO-DMT (Schmid and Bohn 2010). Moreover, measurements of other signal transduction molecules in vitro, including 5-HT_{2A}–$G\alpha_{i/o}$-elicited arachidonic acid release (phospholipase A activation) do not reveal unique signaling properties of psychedelics, and like the phospholipase C-β–inositol phosphate pathway, many psychedelics are partial agonists relative to 5-HT (Kurrasch-Orbaugh et al. 2003a, b; Moya et al. 2007). Gonzalez-Maeso's group has focused on alternative 5-HT_2–$G\alpha_{i/o}$ signaling in vivo. They show that serotonergic psychedelics, but not lisuride (considered a non-hallucinogenic 5-HT_{2A} agonist), alter gene expression through pertussis toxin-sensitive $G\alpha_{i/o}$ signaling (Gonzalez-Maeso et al. 2007). It should be noted, however, that gene expression changes peak after the induction of behavioral responses, thus, likely do not cause them (Gonzalez-Maeso et al. 2007). Nevertheless, in vitro studies show that pertussis toxin decreases DOI- and LSD-elicited inositol phosphate production and abolishes their potentiation of Erk1,2 phosphorylation but does not impact lisuride and ergotamine responses (Karaki et al. 2014).

These observations show that our understanding of serotonergic psychedelic mechanisms is unripe, and creative studies need to be conducted. For example, clozapine (Clozaril®), an inverse agonist at the 5-HT_{2A}–$G\alpha_q$–inositol phosphate pathway (Egan et al. 1998b) and arguably the most effective antipsychotic (Wenthur and Lindsley 2013) activates 5-HT_{2A}-mediated AKT phosphorylation, an effect blocked by the selective 5-HT_{2A} antagonist M100907 (Schmid et al. 2014). Also, like other 5-HT_{2A} agonists, clozapine causes 5-HT_{2A} receptor internalization, whereas ketanserin does not (Raote et al. 2013). Recent studies also revealed that psilocin is a 5-HT reuptake inhibitor, i.e., blocks the serotonin transporter, SERT (Blough et al. 2014; Rickli et al. 2016). Also, classic psychedelics can activate TAAR1 and sigma-1 receptors (Bunzow et al. 2001; Fontanilla et al. 2009; Simmler et al. 2016). Intriguingly, 2C-B, traditionally viewed as a selective 5-HT_2 agonist, has an inhibitory potency at SERT similar to MDMA (Montgomery et al. 2007); similar effects were observed with DIPT (Rickli et al. 2016). Moreover, DMT causes serotonin efflux from SERT with efficacies similar to MDMA (Rickli et al. 2016).

Despite micromolar 5-HT_{1A} affinities (Rickli et al. 2015b), *N*-benzylphenethylamines retain potent psychedelic effects. Also, benzofurans, such as 5-APB and 6-APB, are potent and efficacious 5-HT_{2B} agonists but have very low potency at 5-HT_{2A} receptors. They also stimulate efflux of DA and 5-HT and have activity at TAAR1 receptors (Iversen et al. 2013; Rickli et al. 2015a), but anecdotal reports note that psychedelic effects are relatively minor compared to classic psychedelics. These observations provide further credence that the 5-HT_{2A} receptor, but not 5-HT_{1A}, 5-HT_{2B}, TAAR1, or monoamine transporters, initiates psychedelic effects. These and other proteins may modulate psychedelic effects. The 5-HT_{1A} partial agonist (and β-adrenergic antagonist (Hoffmann et al. 2004)), pindolol, for example, strongly potentiates psychedelic effects of DMT (Strassman 2001). In conclusion, despite the central role of 5-HT_{2A} receptors in producing psychedelic effects, we are still lurking in a fuzzy arena regarding mechanisms after the receptor binding event.

3.3 X-Ray Crystallography

Molecular modeling, molecular dynamics simulations, medicinal chemistry, and molecular pharmacology studies, employing point mutations of 5-HT_{2A} receptor amino acids that alter ligand–receptor molecular interactions, help illustrate how serotonergic psychedelics interact with the 5-HT_{2A} receptor (Braden and Nichols 2007; Braden et al. 2006; Chambers and Nichols 2002; Choudhary et al. 1995; Isberg et al. 2011; Perez-Aguilar et al. 2014). The new gold standard, however, for deciphering the precise fit of a ligand at a receptor and the conformation(s) of the receptor it stabilizes is to isolate crystals of the receptor with the ligand bound, and to develop atomic-level resolution (low ångström, i.e., <3.0 Å) crystallographic images of them. Numerous, resolved GPCR crystal structures with agonists, antagonists, or inverse agonists bound have been reported recently (Hua et al. 2016, 2017; Thal et al. 2016; Wang et al. 2017) and are poised to quickly evolve the structure-based drug discovery process (Ranganathan et al. 2017).

The 5-HT_{2B} crystal structure with LSD bound revealed how a classic psychedelic precisely interacts with a 5-HT_2 receptor and delivered a putative snapshot of a psychedelic receptor conformation (Wacker et al. 2017). LSD binds in the orthosteric pocket of 5-HT_{2B}, which is characterized by many hydrophobic side chains from residues in transmembranes III, V, VI, and VII; recent mutagenesis studies and a resolved 5-HT_{2C} crystal structure confirm that this pocket is also quite similar in the 5-HT_{2C} receptor (Canal et al. 2011; Cordova-Sintjago et al. 2014; Liu et al. 2017; Peng et al. 2018). The basic nitrogen of the ergoline system forms a salt bridge with aspartic acid residue D135 in transmembrane III—this critical interaction is conversed across aminergic GPCRs (Katritch et al. 2013). LSD's ergoline system has aromatic interactions with F340 and F341 in transmembrane VI, and its indole nitrogen hydrogen bonds with G221 in transmembrane V. LSD binds differently than ergoline, as ergoline's indole nitrogen forms a distinct hydrogen bond with T140 in 5-HT_{2B}'s transmembrane VII (Wang et al. 2013), a bond not seen with LSD–5-HT_{2B}. Mutating the homologous residue in 5-HT_{2C} to alanine significantly reduces 5-HT's affinity and agonist potency to stimulate 5-HT_{2C}–phosphoinositide hydrolysis (Liu et al. 2017).

LSD also interacts with the previously described extended binding pocket of 5-HT_{2B} (Wang et al. 2013); specifically, its ethyl groups interact with residues W131 and L132 in transmembrane III, and L362 in transmembrane VII. These interactions may be key to LSD's psychedelic effects, as molecular modeling and ligand docking at 5-HT_{2A} appear to show that they persist for LSD but are lost with LSA, which lacks the diethylamide moiety of LSD (Wacker et al. 2017). (Though, L132 in 5-HT_{2B} is I132 in 5-HT_{2A}.) *TiHKAL* (Shulgin and Shulgin 1997) describes an LSA self-report from Albert Hoffman (who discovered LSD):

> An i.m. administration of a 500 microgram dose led to a tired, dreamy state with an inability to maintain clear thoughts. After a short period of sleep, the effects were gone and normal baseline was recovered within five hours. Other observers have confirmed this clouding of consciousness leading to sleep.

This report illustrates that LSA is substantially less psychoactive than LSD. Since it is distinct from LSD only in its lack of the diethylamide moiety, the hydrophobic interactions between the diethyl structures and 5-HT_{2A} may be central to LSD's psychedelic effects via 5-HT_{2A}. LSA is as efficacious as LSD at activating 5-HT_{2A}–$G\alpha_q$-mediated calcium flux and 5-HT_{2A}-mediated β-arrestin2 recruitment, but 1–2 orders of magnitude less potent (Wacker et al. 2017).

The LSD–5-HT_{2B} structure has similar conformational features as active GPCRs, but with a bias towards a β-arrestin2 state; this was subsequently confirmed in functional assays that show LSD has a potency bias towards β-arrestin2 versus $G\alpha_q$ signaling (Wacker et al. 2013, 2017). These data may suggest that 5-HT_{2A}–β-arrestin2 signaling contributes significantly to psychedelic effects. However, knockout of β-arrestin2 does not reduce the 5-HT_{2A}-mediated head-twitch response caused by two psychedelics, DOI and 5-MeO-DMT (Schmid and Bohn 2010; Schmid et al. 2008). Resolution of 5-HT_{2A} crystal structures with serotonergic psychedelics from the phenethylamine and tryptamine classes may reveal commonalities regarding ligand–receptor interactions and receptor conformations that may ignite the psychedelic experience (Nichols 2017). However, crystal structures (~3 Å) of the β2-adrenergic receptor with an antagonist or inverse agonist bound did not reveal robust conformational differences, suggesting ligands with different functional properties may alter receptor dynamics more so than receptor structure (Wacker et al. 2010). Because ligand–receptor signaling is a dynamic, spatial–temporal process (Grundmann and Kostenis 2017), advanced molecular dynamics (Saleh et al. 2017) describing ligand–receptor–G-protein-binding sequences may be needed to reveal the subtleties of psychedelics acting at 5-HT_{2A} receptors.

4 Preclinical Animal Models

4.1 Head-Twitch Response

The psychedelic-induced head-twitch response in rodents was first reported in 1956 (Keller and Umbreit 1956; Winter and Flataker 1956), and validation of the assay was provided in 1967 (Corne and Pickering 1967). Since then, numerous groups have shown that serotonergic psychedelics elicit the behavior via a 5-HT_{2A} mechanism. Tested psychedelics include LSD, psilocybin, psilocin, DMT, mescaline, 5-MeO-DMT, 5-MeO-DIPT, DPT, 2C-T-7, DOM, DOB, DOI, 2C-I, and several new phenethylamines, tryptamines, and lysergamides (Brandt et al. 2016, 2017a, b; Canal and Morgan 2012; Corne and Pickering 1967; Fantegrossi et al. 2005, 2006, 2008; Halberstadt and Geyer 2013, 2014, 2017; Halberstadt et al. 2011; Moya et al. 2007; Nichols et al. 2015).

Mice display a head-twitch response—observed as rapid, lateral rotations of the head (Halberstadt and Geyer 2013)—commencing within a few minutes after peripheral administration of a psychedelic, and with phenylisopropylamines, the response peaks in about 10 min and persists for at least 2 h (Canal and Morgan

2012). 5-HT_{2A} antagonists block the head-twitch in mice, rats, and the least shrew (Canal and Morgan 2012; Darmani et al. 1994; Halberstadt and Geyer 2017; Schreiber et al. 1995), whether administered before or after induction of the head-twitch response, as observed in C57BL/6J mice (Canal et al. 2013a). Serotonergic psychedelics do not elicit a head-twitch response in 5-HT_{2A} knockout mice, but restoration of 5-HT_{2A} receptors to cortical neurons restores the ability of psychedelics to elicit the response (Gonzalez-Maeso et al. 2007).

What has made the head-twitch response particularly attractive for studying serotonergic psychedelic mechanisms is that lisuride does not produce it in mice (Gonzalez-Maeso et al. 2007; Halberstadt and Geyer 2013). Lisuride does, however, elicit a head-twitch response in the least shrew (Darmani et al. 1994), which appears a particularly sensitive species; (±)-DOI, 0.63 mg/kg, elicits an average of 263 head-twitches in 30 min, whereas lisuride, 1.25 mg/kg, elicits an average of 49 head-twitches in the same time period (Darmani et al. 1994). As a comparison, adult, male C57BL/6J mice exhibit about 30–40 head-twitches in a 10-min period after (±)-DOI, 1 mg/kg (Canal and Morgan 2012; Halberstadt and Geyer 2013). Furthermore, lisuride is a low-efficacy 5-HT_{2A} agonist (Berg et al. 1998) and appears to be distinguished from other 5-HT_{2A} agonists, especially, by its weak potency and efficacy at stimulating intracellular calcium mobilization; for example, its efficacy relative to 5-HT is ~49%, whereas LSD's efficacy is ~85% (Cussac et al. 2008). A recent study reports a significant correlation between the potencies of phenethylamines and tryptamines to activate 5-HT_{2A}-mediated calcium mobilization and their potencies to elicit the head-twitch response (Nichols et al. 2015). Calcium mobilization can be independent of the phospholipase C-β–IP_3 receptor pathway, and this signaling pathway does not appear to control all psychoactive effects, as noted above. Thus, these results provide an intriguing possibility that IP_3 receptor-independent calcium mobilization may uniquely contribute to psychedelic effects.

Nevertheless, there are clear false positives in the head-twitch assay. The 5-HT releaser *d*-fenfluramine is non-hallucinogenic but elicits the head-twitch in mice (Darmani 1998)—fenfluramine does, however, displace the 5-HT_{2A} agonist radioligand [^{11}C]Cimbi-36 from binding sites in primate brains (Yang et al. 2017), suggesting that the head-twitch may be sensitive to drugs that indirectly stimulate 5-HT_{2A} receptors. False negatives also show that it has questionable translational validity. To illustrate, anecdotal reports note that cannabis—psychoactive due to THC partial agonist activity at cannabinoid 1 (CB1) receptors—potentiates serotonergic psychedelic effects in humans. However, numerous compounds that stimulate CB1 receptors, including THC, eliminate the head-twitch elicited by DOI, whereas the CB1 inverse agonist, SR 141716A (Rimonibant®), can elicit the head-twitch (Ceci et al. 2015; Darmani 2001; Darmani et al. 2003; Darmani and Pandya 2000; Egashira et al. 2004, 2011).

The psychedelic-elicited head-twitch response can be modulated, albeit mostly suppressed, by a number of compounds that target receptors other than 5-HT_{2A}, including 5-HT_{2C}, 5-HT_{1A}, glutamate NMDA, AMPA and mGluR2, α-adrenergic, and dopamine D2 receptors, and others; regardless of the modulatory effect,

selective 5-HT_{2A} blockade abolishes the head-twitch (Canal and Morgan 2012). Thus, the 5-HT_{2A} receptor appears to functionally interact with numerous neurotransmitter systems. Some mice including C57BL/6J mice naturally exhibit the head-twitch at low levels. For example, we have observed scores of adult, male, drug-naïve C57BL/6J mice, and each exhibits ~2–5 robust head-twitches in 10 min (Canal et al. 2013a); others report similar observations (Halberstadt and Geyer 2013). Interestingly, it too is blocked by selective 5-HT_{2A} antagonism (Canal et al. 2013a), corroborating the conclusion that the 5-HT_{2A} receptor, regardless of whether it is activated by a psychedelic or by endogenous mechanisms, mediates the head-twitch response.

Many novel lysergamides including 1P-LSD, LSZ, and AL-LAD elicit a dose-dependent head-twitch in C57BL/6J mice (Brandt et al. 2016, 2017b). LSM-775 does not produce a head-twitch in C57BL/6J mice unless they are pretreated with a 5-HT_{1A} antagonist (Brandt et al. 2017a). LSM-775 also appears to lack psychedelic effects in humans, which is peculiar, as other psychedelics are potent and efficacious 5-HT_{1A} agonists, including LSD (Pauwels et al. 1993), and as noted above, the 5-HT_{1A} partial agonist, pindolol, potentiates the psychedelic effects of DMT (Strassman 2001). Moreover, the potent 5-HT_{1A} agonist (±)-8-OH-DPAT enhances the stimulus effects of DOM (Glennon 1991). However, 5-HT_{1A} agonists including both enantiomers of 8-OH-DPAT potently block the head-twitch response elicited by some, but not all, serotonergic psychedelics (Canal et al. 2015; Dursun and Handley 1993; Goodwin and Green 1985), and the 5-HT_{1A} antagonist/dopamine D2/D3 agonist S(-)-UH-301 alone can elicit a head-twitch response in mice (Darmani and Reeves 1996).

4.2 Drug Discrimination

The two-lever, appetitive, drug discrimination task is an authoritative, preclinical tool for determining psychedelic drug mechanisms in vivo. A food-motivated animal is trained in an operant task, under one of several reinforcement schedules, to press one lever for a food reward when it is under the influence of a training drug, and the other lever when it is not. Once the animal clearly shows it can discriminate or recognize the effects of the drug by successfully pressing the correct lever on repeated trials, e.g., with accuracy $\geq$80% (typically requiring several weeks of training), it can then be treated with test drugs to observe whether they substitute (partially to fully) for the training drug, or when co-administered with the training drug, suppress (partially to fully) its discriminative stimulus effects. For example, if a test drug causes animals to make $\geq$80% of their responses on the lever associated with the training drug, investigators infer that the two compounds produce similar subjective or stimulus effects. Conversely, if a test drug causes animals to make $\leq$20% of their responses on the lever associated with the training drug, investigators infer that the test drug does not produce subjective effects that are like the training drug. Even if the test drug has discriminative effects, if they are unlike the training drug, animals typically default to responding by pressing the vehicle-associated

lever. If a test drug causes animals to split their responses between the levers, then investigators infer that it has effects somewhat similar to the training drug (Glennon and Young 2011). Like the head-twitch procedure, drug discrimination can provide information regarding drug pharmacokinetics and pharmacodynamics (e.g., drug onset, duration, potency, and mechanism of action), and drug discrimination has high predictive validity, i.e., it translates well to human subjects. Importantly, the drug discrimination procedure distinguishes psychoactive drugs from various classes, and germane here, primates trained using two-choice drug discrimination unmistakably distinguish different types of hallucinogens, e.g., κ-opioid agonist hallucinogens from NMDA antagonist hallucinogens and from serotonergic psychedelics (Butelman et al. 2010). For a detailed study of the drug discrimination procedure, including its utility and arguments regarding its superiority relative to other behavioral assays, see Glennon and Young (2011).

Early drug discrimination studies with rodents that showed 5-HT_{2A} antagonists reduce the stimulus effects of diverse serotonergic psychedelics (Glennon 1992; Glennon et al. 1983) provided commanding evidence that 5-HT_{2A} receptor activation is their unifying and common mechanism. This evidence was corroborated by studies employing DOx psychedelics, which are 5-HT_2 selective agonists, as test drugs that substitute for LSD (Fiorella et al. 1995). Other studies confirmed that selective blockade of 5-HT_{2A} receptors, i.e., by M100907 (Kehne et al. 1996; Palfreyman et al. 1993), occludes the discriminative stimulus effects of some serotonergic psychedelics in rats and primates (Li et al. 2007, 2009a; May et al. 2009; Schreiber et al. 1994).

Recent studies employing drug discrimination in rats show that novel psychedelics including 25I-, 25B-, and 25C-NBOMe, and 5-MeO-DALT fully substitute for DOM; interestingly, the NBOMe drugs also substitute for MDMA, but 5-MeO-DALT does not (Gatch et al. 2017). This study and others illustrate the utility of drug discrimination assays to differentiate unique effects of different serotonergic psychedelics. The selective 5-HT_{2C} antagonist SB242084 (1 mg/kg) blocks ~70% of DIPT lever responding but does not affect the DMT discriminative stimulus (Carbonaro et al. 2015). Intriguingly, also from this study, M100907 does not completely block the discriminative stimulus effects of DIPT but does completely block DMT's effects. Also, 2C-T-7 only partially substitutes for psilocybin and LSD (~40% and ~75%, respectively) in rats at a dose (1 mg/kg) that appears to be maximal for eliciting the head-twitch response in mice (Fantegrossi et al. 2005; Winter et al. 2007). Also from these studies, psilocybin at 1 mg/kg partially (~50%) substitutes for LSD, and M100907 completely blocks the substitution. M100907 (0.5 mg/kg) also completely blocks 2C-T-7's stimulus effects. M100907 (0.2 mg/kg) partially (~40%) blocks psilocybin's stimulus effects, whereas (±)-8-OH-DPAT mostly (~80%) blocks them; the effect of (±)-8-OH-DPAT is not blocked by the selective 5-HT_{1A} antagonist, WAY-100635 (0.2 mg/kg), nor does this compound on its own (up to 0.6 mg/kg) alter the stimulus effects of psilocybin. In humans, however, the non-hallucinogenic 5-HT_{1A} receptor partial agonist buspirone reduces psilocybin-induced simple and complex hallucinations (Pokorny et al. 2016). Clearly, different mechanisms contribute to the stimulus

effects of different serotonergic psychedelics, though, again, like the head-twitch response, 5-HT_{2A} receptors appear to control a significant portion of the effects, with some exceptions. This translates well to humans, as the psychedelic effects of both psilocybin and LSD are blocked by a fairly selective 5-HT_{2A} antagonist, ketanserin—though see Sect. 3.6.

Similar to the head-twitch model, compounds targeting receptors other than 5-HT_{2A} modulate the discriminative stimulus effects of serotonergic psychedelics, and false positives, false negatives, and misunderstood results have emerged (Benneyworth et al. 2005; Reissig et al. 2005; Winter 2009). For example, lisuride substitutes for a number of serotonergic psychedelics in the two-lever drug discrimination paradigm; however, this can be overcome by training animals to discriminate two training drugs and vehicle. Thus, when animals are trained to discriminate lisuride, LSD, and vehicle, lisuride does not substitute for LSD (Appel et al. 2004). Regarding false negative responses, LSD, DOM, and DOI substitute for fenfluramine (Glennon 1991; McCreary et al. 2003). DOI, at 1 mg/kg, engenders rats to respond to the (±)-fenfluramine (2 mg/kg)-associated lever ~73% of the time, and this effect is completely blocked by the 5-HT_{2B}/5-HT_{2C} inverse agonist, SB206553 (1 mg/kg); interestingly, M100907 also dose-dependently attenuates (but does not fully block) the effect (McCreary et al. 2003). In C57BL/6J mice, SB206553 suppresses the DOI-elicited head-twitch response (Canal et al. 2010, 2013a), suggesting that 5-HT_{2B} or 5-HT_{2C} receptors may contribute to DOI's effects. Often overlooked, M100907 has relevant affinity at mouse and human 5-HT_{2C} receptors (K_i ~40–100 nM (Canal et al. 2013a); PDSP certified), where it acts as a 5-HT_{2C} inverse agonist with potency and efficacy similar to the well-appreciated 5-HT_{2C} inverse agonist, clozapine (Herrick-Davis et al. 2000; Kehne et al. 1996; Navailles et al. 2006; Rauser et al. 2001).

MDMA nearly (~80%) substitutes for DMT and similarly nearly (~80%) substitutes for methamphetamine in two-lever drug discrimination assays (Gatch et al. 2009). mGluR2 activation, which suppresses DOB- and DOI-elicited head-twitches, fails to alter discriminative stimulus effects of DIPT, DMT, and DOB in mice (Benneyworth et al. 2008; Carbonaro et al. 2015; Griebel et al. 2016). However, the prolonged training regimen and multidosing of serotonergic psychedelics that drug discrimination requires may cause functional changes in mGluR2 receptors that mask effects of mGluR2 activation (Benneyworth et al. 2008). Interestingly, the 5-HT_3 antagonist/5-HT_4 agonist zacopride potently and efficaciously reduces the discriminative stimulus properties of DOM (and MDMA) (Glennon et al. 1992). Finally, unique serotonergic psychedelics may produce different stimulus properties depending on their training dose or depending on when the training drug is administered prior to engaging in the associative learning task. For example, LSD's stimulus effects are 5-HT_{2A} mediated 30 min after its administration but appear to be dopamine D2 receptor mediated 90 min after administration (Marona-Lewicka et al. 2005). Interpretation of results from drug discrimination studies should consider different targets engaged by training drugs administered at different doses and at different times.

5 Measuring Localized and System Effects in the Brain

Recent, clinical neuroimaging studies have revealed serotonergic psychedelic effects that may explain not only neural perturbations that underlie visual hallucinations, e.g., decreases in alpha oscillations in visual cortex (Carhart-Harris et al. 2016; Kometer et al. 2013; Kometer and Vollenweider 2016), but also psychotherapeutic benefits of serotonergic psychedelics. One hypothesis, supported by recent observations, is that psychedelics have entropic effects on cortical activity, razing entrenched functional connectivity while sprouting new patterns of connectivity (Carhart-Harris et al. 2014; Petri et al. 2014; Tagliazucchi et al. 2014, 2016); for detailed discussions of this evolving topic, refer to Atasoy et al. (2017), Carhart-Harris et al. (2017), and Viol et al. (2017). Studies show that brains from subjects with treatment-resistant depression exhibit hyperactivity (entrenchment) within certain circuits, including the subcallosal cingulate white matter, which has been subsequently targeted by deep-brain stimulation (Mayberg et al. 1997; Riva-Posse et al. 2017). Psilocybin helps relieve treatment-resistant depression (Carhart-Harris et al. 2017), and intriguingly, psilocybin and LSD alter activity within the cingulate cortex and functional connectivity between the cingulate cortex and other brain systems (Carhart-Harris et al. 2012, 2016). These neural perturbations correlate with dissolution of ego boundaries (loss of the self), suggesting the possibility that this psychedelic phenomenon may, in certain individuals, help relieve psychiatric distress (c.f. Griffiths et al. 2016; Ross et al. 2016; Vollenweider 2001).

Preclinical strategies can provide additional spatial and temporal precision and a reductionist understanding of mechanisms which span genetic, molecular, cellular, and neural systems. Approaches to measure localized effects include direct brain injections, via drug infusions through stereotaxically implanted cannulae as well as systemic injections followed by measurements of changes in neurochemicals in discrete neural systems, e.g., via in vivo microdialysis combined with high-performance liquid chromatography and/or liquid chromatography–mass spectrometry or voltammetry detection (Bucher and Wightman 2015; Kennedy 2013). Measurements of electrophysiological effects on distinct brain cell types in distinct brain regions, e.g., via multichannel recordings from precisely implanted electrodes or from brain slice preparations (Du et al. 2017), can provide information about 5-HT_2 effects on cell and network excitability, neurotransmission, and neuroplasticity. Invasive approaches also allow researchers to measure, for example, psychedelic-induced changes in DNA methylation (epigenetics), RNA transcription (gene expression), protein synthesis, and phosphorylation (posttranslational modifications). Serotonergic psychedelics alter the expression of genes that contribute to synaptic plasticity, providing physiological evidence that these compounds cause persistent changes in the brain that may underlie their therapeutic effects (Martin and Nichols 2017). For example, 24-h treatment of 45-day-old, brain organoids with 5-MeO-DMT alters the expression of proteins involved in memory consolidation, cytoskeletal organization, and inflammation, e.g., CaMKII, ephrin-B2, and NF-κB, respectively (Dakic et al. 2017).

Invasive techniques also permit analyses of interactions between receptor targets and other proteins. 5-HT_{2A} and 5-HT_{2C} receptors directly bind to synaptic scaffolding proteins, such as PSD-95 (Becamel et al. 2004; Xia et al. 2003). Also, 5-HT_{2A} and glutamate mGluR2 receptors interact closely, and their interaction may be a key mechanism underlying psychedelic effects (Benneyworth et al. 2007; Delille et al. 2012; Gonzalez-Maeso et al. 2008; Lee et al. 2014; Moreno et al. 2011). Similarly, analyzing gene expression patterns allows researchers to observe which genes the brain expresses in regions where receptor targets of serotonergic psychedelics are expressed. Using the Allen Brain Atlas online resource, one can see that the gene coding for 5-HT_{2A} receptors, *HTR2A*, is expressed in cortical brain regions that overlap very closely with expression of the gene coding for one of the subunits of glutamate NMDA receptors, *NR2A*, which the psychedelic dissociative ketamine targets. This connection fits well with results from PET (^{18}fluorodeoxyglucose) imaging studies that show psilocybin and ketamine produce similar prefrontal cortex–limbic activation patterns in humans (Vollenweider 2001).

5.1 Serotonergic Psychedelics Impact on Neurotransmission

The effects of serotonergic psychedelics on neurotransmission have only been resolved partially. After systemic injections in rodents, serotonergic psychedelics increase glutamate in the cortex and also the ventral tegmentum area (Muschamp et al. 2004; Pehek et al. 2006; Scruggs et al. 2003). They also increase dopamine release in the cortex, but not the nucleus accumbens or the striatum (Di Matteo et al. 2000; Gudelsky et al. 1994; Pehek and Hernan 2015; Pehek et al. 2001, 2006). DOI increases acetylcholine release in the prefrontal cortex and the hippocampus (Nair and Gudelsky 2004; Zhelyazkova-Savova et al. 1997, 1999); also Nair and Gudelsky (2004) show that mescaline increases acetylcholine release in the prefrontal cortex, but not the hippocampus. DOI decreases norepinephrine release in the hippocampus (Done and Sharp 1992), though this study was performed in anesthetized rats. Finally, DOI increases GABA release in the prefrontal cortex (Abi-Saab et al. 1999). Most of the neurochemical effects of compounds in the aforementioned studies were blocked by 5-HT_2 antagonists.

Early studies showed that serotonergic psychedelics increase brain 5-HT levels (Freedman and Giarman 1962; Giarman and Freedman 1965), but few studies since have systematically dissected this effect. One study shows that peripherally administered DOI has no effect on 5-HT release in the prefrontal cortex (Gobert and Millan 1999), yet others show that DOI significantly decreases 5-HT release there, in anesthetized rats (Martin-Ruiz et al. 2001; Wright et al. 1990). Other studies show that direct prefrontal cortex application of DOI increases 5-HT release there (Amargos-Bosch et al. 2004; Bortolozzi et al. 2003; Martin-Ruiz et al. 2001) or has no effect (Wright et al. 1990). Still another shows that DOI decreases 5-HT release from cortical slices while concordantly increasing GABA release; the increase in GABA release caused by DOI is also observed using cortical synaptosome

preparations (Luparini et al. 2004). Finally, direct striatal DOI application increases 5-HT release there (Abellan et al. 2000).

A few recent studies have employed electrophysiological approaches to examine effects on neurotransmission. The 5-HT_{2A} agonist and putative psychedelic, TCB-2, inhibits pyramidal neurons in layer 6 of the prefrontal cortex (Tian et al. 2016); an earlier study showed that 5-HT_2 receptor activation stimulates (presumably) GABA interneurons in layer 2/3 of piriform cortex (Gellman and Aghajanian 1994). 5-HT_2 receptors also increase activity of layer 5 GABAergic interneurons and glutamatergic pyramidal neurons (Aghajanian and Marek 1997; Spindle and Thomas 2014; Weber and Andrade 2010; Zhang and Arsenault 2005). These results align with the robust expression of 5-HT_2 receptors on both GABAergic and glutamatergic neurons in the cortex (Puig et al. 2010; Willins et al. 1997). Finally, a recent study shows that low-dose LSD inhibits dorsal raphe nuclei 5-HT firing, and high-dose LSD additionally decreases firing of ventral tegmentum area dopamine neurons; the former effects are blocked by haloperidol and M100907, and latter effects are blocked by haloperidol, WAY-100635, and the TAAR1 antagonist, EPPTB (De Gregorio et al. 2016).

5.2 Neural Systems Underlying Serotonergic Psychedelic Effects

Elaine Sanders-Bush's group combined brain microinfusions with drug discrimination to directly test the contribution of the anterior cingulate cortex to the discriminative stimulus properties of LSD; they found that local infusions of LSD substitute for systemically administered LSD, and that local infusions of M100907 block LSD's discriminative stimulus properties (Gresch et al. 2007). Similarly, DOI directly infused into the anterior cingulate cortex elicits a head-twitch response, which is blocked by systemic injections of M100907 (Willins and Meltzer 1997). Despite these major findings, few other studies have directly assessed the involvement of other neural systems in serotonergic psychedelic effects (Halberstadt and Geyer 2017). Most information is based on correlation analyses from clinical trials, but these reports support preclinical observations. PET imaging with [^{18}F]altanserin, a 5-HT_{2A} antagonist radioligand, showed that psilocybin's receptor occupancy in the anterior cingulate and medial prefrontal cortices correlates with psychedelic intensity (Quednow et al. 2010). Functional changes in the brain caused by serotonergic psychedelics include activity increases and decreases as well as modulation of interactions within and across cortical regions, between the thalamus and cortex, hippocampus, amygdala, and cortex, and across regions typically not functionally associated, typifying entropic effects of serotonergic psychedelics—some of these changes significantly correlate with distinct psychedelic effects (c.f. Carhart-Harris et al. 2016; Mueller et al. 2017; Palhano-Fontes et al. 2015; Petri et al. 2014; Tagliazucchi et al. 2016).

6 On Ketanserin, Conformations, and Dimerization

Reports from clinical trials conclude that the psychedelic effects of psilocybin and LSD are mediated by 5-HT_{2A} receptors, because they are blocked by ketanserin (40 mg, P.O.), typically viewed as a selective 5-HT_{2A} antagonist (Kometer et al. 2012; Kraehenmann et al. 2017; Preller et al. 2017; Quednow et al. 2012). Haloperidol, typically viewed as a selective dopamine D2 antagonist, is much less effective than ketanserin at blocking psilocybin's effects, but risperidone, an antipsychotic with combined D2/5-HT_2 activity, is as effective as ketanserin (Vollenweider et al. 1998).

Ketanserin, however, at <2 nM concentration labels a site(s) distinct from 5-HT_{2A} receptors in several species, including humans, and in several neural systems, notably the striatum, substantia nigra, and raphe nuclei; in rats, this site appears to control the release of the dopamine metabolite DOPAC from dopamine nerve terminals (Leysen et al. 1987; Lopez-Gimenez et al. 1998; Pazos et al. 1987). Recently, Glennon commented on ketanserin's off-target effects (Glennon 2017):

> The lack of ketanserin's selectivity for 5-HT_2 receptors over some other receptors, notably histamine receptors and certain adrenoceptors, was a drawback when brain homogenates were being employed as the receptor source.

Importantly, M100907, viewed as one of the most selective, commercially available 5-HT_{2A} antagonists, only effects ~50% of ketanserin-appropriate lever responding in rats trained to discriminate ketanserin from saline; prazosin, an α1-adrenergic receptor antagonist, combined with M100907 causes full substitution (Li et al. 2009b). These observations suggest that ketanserin blocks α1-adrenergic receptors in vivo, which produces a subjectively recognizable effect. Importantly, α1-adrenergic receptors co-localize with 5-HT_{2A} receptors in the prefrontal cortex (Santana et al. 2013), suggesting they may functionally interact in vivo. In addition to α1-adrenergic receptors, ketanserin also has relevant (off-target) affinity at human H1, 5-HT_{1D}, 5-HT_{1F}, and 5-HT_{2C} receptors (Boess and Martin 1994; Bonhaus et al. 1995; Domenech et al. 1997; Ghoneim et al. 2006), and 5-HT_{2C} receptors also co-localize with 5-HT_{2A} receptors in certain parts of the cortex (Santana and Artigas 2017).

When considering haloperidol's inefficacy at blocking psychedelic effects of psilocybin in humans and DOI's discriminative stimulus properties in rats (Schreiber et al. 1994; Vollenweider et al. 1998), it should be noted that haloperidol has no activity at 5-HT_{2C} receptors but possesses relevant affinity at 5-HT_{2A} receptors (Herrick-Davis et al. 2000; Kroeze et al. 2003; Leysen et al. 1993; Rauser et al. 2001; Richelson and Souder 2000). Conversely, ketanserin and risperidone are efficacious 5-HT_{2C} inverse agonists that also have high affinity at 5-HT_{2A} (Hartman and Northup 1996; Herrick-Davis et al. 2000; Richelson and Souder 2000). Moreover, in competition binding assays, risperidone and ketanserin recognize two 5-HT_{2A} receptor sites (defined by two slopes in the displacement curves) labeled with [^{3}H](±)-DOB, whereas haloperidol recognizes only one site. In functional

assays, each drug antagonizes 5-HT_{2A}-stimulated inositol phosphate production and arachidonic acid release, but risperidone and ketanserin antagonize the latter in a biphasic manner similar to their binding characteristics (Brea et al. 2009). The authors suggest that the pharmacological differences are due to differential recognition of 5-HT_{2A} receptor homodimers – that risperidone and ketanserin bind 5-HT_{2A} homodimers, but haloperidol does not. It is, therefore, intriguing to consider that haloperidol may be ineffective at blocking psilocybin's psychedelic effects, because it may not block putative 5-HT_{2A} homodimers activated by psilocybin, whereas ketanserin and risperidone may. Risperidone potently blocks 5-HT_2 mediated activation of (presumably) GABAergic interneurons in piriform cortex, whereas haloperidol, up to 10 μM, only weakly blocks these effects (Gellman and Aghajanian 1994). Alternatively, since haloperidol has negligible affinity at 5-HT_{2C} receptors, by extension, it may not be able to bind 5-HT_{2A}–5-HT_{2C} heterodimers, which were recently observed in vitro and in brains (Moutkine et al. 2017). Co-activation of 5-HT_{2A} and 5-HT_{2C} receptors has been postulated as a mechanism underlying serotonergic psychedelic effects (Burris et al. 1991; Canal et al. 2010).

Finally, a couple of recent clinical trials suggest that 5-HT_{2A} receptor activation may not be necessary or sufficient to produce all of the psychoactive effects of serotonergic psychedelics. Ketanserin (40 mg, P.O.) does not entirely block the effects of an ayahuasca brew (containing DMT, harmine, harmaline, and THH); notably, it does not block "modifications in time perception," feeling "high," or "visions" (Valle et al. 2016). This report suggests that there is a mechanism other than 5-HT_{2A} at large, and the authors propose to further investigate sigma-1 receptor activation (Valle et al. 2016). Alternatively, ketanserin may not block unique 5-HT_2 receptor ensembles and/or conformations stabilized by ayahuasca. [^{125}I]DOI autoradiography studies show that 5-HT_{2A} and 5-HT_{2C} receptors exist in multiple conformations across neural systems in the rat brain, and ketanserin and M100907 have different affinities at them (Lopez-Gimenez et al. 2013). Collectively, then, data suggest that different 5-HT_2 antagonists may selectively block unique 5-HT_2 receptor homodimer or heterodimer ensembles and/or receptor conformations—it remains unclear which are the mechanistic targets of psychedelics. Clearly, more serotonergic psychedelics research aimed at elucidating their mechanisms needs to be conducted.

7 Conclusions

Recent experiments have begun to unveil neuropsychological mechanisms of serotonergic psychedelics, and clinical studies with ketanserin support a central role for serotonin 5-HT_{2A} receptors in producing psychedelic effects. There remains fertile ground, however, for much more discovery. Unknown are the cellular signal transduction conduits of 5-HT_2 receptor activation, the 5-HT_2 receptor–protein interactions, and the neural circuits and neurochemical processes within those circuits, that produce distinct psychedelic effects, e.g., "oceanic boundlessness" or "visionary restructuralization" (Dittrich 1998). Also mysterious are the mechanisms

underlying unique psychoactive effects engendered by unique psychedelics. Indeed, it is unclear whether all serotonergic psychedelics can induce psychedelic effects matching those of LSD—experiencing oneness with the universe or an all-encompassing unity, transcending time and space, tapping into the unconscious or experiencing archetypes, and an ethereal, positive, overwhelming luminescent, mental state filled with awe and profound philosophical, spiritual, or religious meaning that is ineffable with words (Pahnke et al. 1970; Pahnke and Richards 1966). Future discoveries will weave together reductionist and emergentist points of view to construct lucid neurobiological pictures illuminating how serotonergic psychedelics work.

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References

Abellan MT, Martin-Ruiz R, Artigas F (2000) Local modulation of the 5-HT release in the dorsal striatum of the rat: an in vivo microdialysis study. Eur Neuropsychopharmacol 10:455–462

Abi-Saab WM, Bubser M, Roth RH, Deutch AY (1999) 5-HT2 receptor regulation of extracellular GABA levels in the prefrontal cortex. Neuropsychopharmacology 20:92–96

Acuna-Castillo C, Villalobos C, Moya PR, Saez P, Cassels BK, Huidobro-Toro JP (2002) Differences in potency and efficacy of a series of phenylisopropylamine/phenylethylamine pairs at 5-HT(2A) and 5-HT(2C) receptors. Br J Pharmacol 136:510–519

Aghajanian GK, Marek GJ (1997) Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. Neuropharmacology 36:589–599

Amargos-Bosch M, Bortolozzi A, Puig MV, Serrats J, Adell A, Celada P, Toth M, Mengod G, Artigas F (2004) Co-expression and in vivo interaction of serotonin1A and serotonin2A receptors in pyramidal neurons of prefrontal cortex. Cereb Cortex 14:281–299

Appel JB, West WB, Buggy J (2004) LSD, 5-HT (serotonin), and the evolution of a behavioral assay. Neurosci Biobehav Rev 27:693–701

Atasoy S, Roseman L, Kaelen M, Kringelbach ML, Deco G, Carhart-Harris RL (2017) Connectome-harmonic decomposition of human brain activity reveals dynamical repertoire re-organization under LSD. Sci Rep 7:17661

Barclay Z, Dickson L, Robertson DN, Johnson MS, Holland PJ, Rosie R, Sun L, Fleetwood-Walker S, Lutz EM, Mitchell R (2011) 5-HT2A receptor signalling through phospholipase D1 associated with its C-terminal tail. Biochem J 436:651–660

Bécamel C, Gavarini S, Chanrion B, Alonso G, Galeotti N, Dumuis A, Bockaert J, Marin P (2004) The serotonin 5-HT2A and 5-HT2C receptors interact with specific sets of PDZ proteins. J Biol Chem 279:20257–20266

Bécamel C, Berthoux C, Barre A, Marin P (2017) Growing evidence for heterogeneous synaptic localization of 5-HT2A receptors. ACS Chem Neurosci 8:897–899

Beliveau V, Ganz M, Feng L, Ozenne B, Hojgaard L, Fisher PM, Svarer C, Greve DN, Knudsen GM (2017) A high-resolution in vivo atlas of the human brain's serotonin system. J Neurosci 37:120–128

Benneyworth MA, Smith RL, Barrett RJ, Sanders-Bush E (2005) Complex discriminative stimulus properties of (+)lysergic acid diethylamide (LSD) in C57Bl/6J mice. Psychopharmacology 179:854–862

Benneyworth MA, Xiang Z, Smith RL, Garcia EE, Conn PJ, Sanders-Bush E (2007) A selective positive allosteric modulator of metabotropic glutamate receptor subtype 2 blocks a hallucinogenic drug model of psychosis. Mol Pharmacol 72:477–484

Benneyworth MA, Smith RL, Sanders-Bush E (2008) Chronic phenethylamine hallucinogen treatment alters behavioral sensitivity to a metabotropic glutamate 2/3 receptor agonist. Neuropsychopharmacology 33:2206–2216

Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P, Clarke WP (1998) Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. Mol Pharmacol 54:94–104

Best AR, Regehr WG (2008) Serotonin evokes endocannabinoid release and retrogradely suppresses excitatory synapses. J Neurosci 28:6508–6515

Blough BE, Landavazo A, Decker AM, Partilla JS, Baumann MH, Rothman RB (2014) Interaction of psychoactive tryptamines with biogenic amine transporters and serotonin receptor subtypes. Psychopharmacology 231:4135–4144

Boess FG, Martin IL (1994) Molecular biology of 5-HT receptors. Neuropharmacology 33:275–317

Bonhaus DW, Bach C, DeSouza A, Salazar FH, Matsuoka BD, Zuppan P, Chan HW, Eglen RM (1995) The pharmacology and distribution of human 5-hydroxytryptamine2B (5-HT2B) receptor gene products: comparison with 5-HT2A and 5-HT2C receptors. Br J Pharmacol 115:622–628

Bortolozzi A, Amargos-Bosch M, Adell A, Diaz-Mataix L, Serrats J, Pons S, Artigas F (2003) In vivo modulation of 5-hydroxytryptamine release in mouse prefrontal cortex by local 5-HT(2A) receptors: effect of antipsychotic drugs. Eur J Neurosci 18:1235–1246

Braden MR, Nichols DE (2007) Assessment of the roles of serines 5.43(239) and 5.46(242) for binding and potency of agonist ligands at the human serotonin 5-HT2A receptor. Mol Pharmacol 72:1200–1209

Braden MR, Parrish JC, Naylor JC, Nichols DE (2006) Molecular interaction of serotonin 5-HT2A receptor residues Phe339(6.51) and Phe340(6.52) with superpotent *N*-benzyl phenethylamine agonists. Mol Pharmacol 70:1956–1964

Brandt SD, Kavanagh PV, Westphal F, Stratford A, Elliott SP, Hoang K, Wallach J, Halberstadt AL (2016) Return of the lysergamides. Part I: Analytical and behavioural characterization of 1-propionyl-d-lysergic acid diethylamide (1P-LSD). Drug Test Anal 8:891–902

Brandt SD, Kavanagh PV, Twamley B, Westphal F, Elliott SP, Wallach J, Stratford A, Klein LM, McCorvy JD, Nichols DE, Halberstadt AL (2017a) Return of the lysergamides. Part IV: Analytical and pharmacological characterization of lysergic acid morpholide (LSM-775). Drug Test Anal. https://doi.org/10.1002/dta.2222

Brandt SD, Kavanagh PV, Westphal F, Elliott SP, Wallach J, Colestock T, Burrow TE, Chapman SJ, Stratford A, Nichols DE, Halberstadt AL (2017b) Return of the lysergamides. Part II: Analytical and behavioural characterization of N6 -allyl-6-norlysergic acid diethylamide (AL-LAD) and (2'S,4'S)-lysergic acid 2,4-dimethylazetidide (LSZ). Drug Test Anal 9:38–50

Brea J, Castro M, Giraldo J, Lopez-Gimenez JF, Padin JF, Quintian F, Cadavid MI, Vilaro MT, Mengod G, Berg KA, Clarke WP, Vilardaga JP, Milligan G, Loza MI (2009) Evidence for distinct antagonist-revealed functional states of 5-hydroxytryptamine(2A) receptor homodimers. Mol Pharmacol 75:1380–1391

Bucher ES, Wightman RM (2015) Electrochemical analysis of neurotransmitters. Annu Rev Anal Chem (Palo Alto, Calif) 8:239–261

Bunzow JR, Sonders MS, Arttamangkul S, Harrison LM, Zhang G, Quigley DI, Darland T, Suchland KL, Pasumamula S, Kennedy JL, Olson SB, Magenis RE, Amara SG, Grandy DK (2001) Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. Mol Pharmacol 60:1181–1188

Burris KD, Breeding M, Sanders-Bush E (1991) (+)Lysergic acid diethylamide, but not its nonhallucinogenic congeners, is a potent serotonin 5HT1C receptor agonist. J Pharmacol Exp Ther 258:891–896

Butelman ER, Rus S, Prisinzano TE, Kreek MJ (2010) The discriminative effects of the kappa-opioid hallucinogen salvinorin A in nonhuman primates: dissociation from classic hallucinogen effects. Psychopharmacology 210:253–262

Canal CE, Morgan D (2012) Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: a comprehensive history, a re-evaluation of mechanisms, and its utility as a model. Drug Test Anal 4:556–576

Canal CE, Olaghere da Silva UB, Gresch PJ, Watt EE, Sanders-Bush E, Airey DC (2010) The serotonin 2C receptor potently modulates the head-twitch response in mice induced by a phenethylamine hallucinogen. Psychopharmacology 209:163–174

Canal CE, Cordova-Sintjago TC, Villa NY, Fang LJ, Booth RG (2011) Drug discovery targeting human 5-HT(2C) receptors: residues S3.36 and Y7.43 impact ligand-binding pocket structure via hydrogen bond formation. Eur J Pharmacol 673:1–12

Canal CE, Booth RG, Morgan D (2013a) Support for 5-HT2C receptor functional selectivity in vivo utilizing structurally diverse, selective 5-HT2C receptor ligands and the 2,5-dimethoxy-4-iodoamphetamine elicited head-twitch response model. Neuropharmacology 70:112–121

Canal CE, Cordova-Sintjago T, Liu Y, Kim MS, Morgan D, Booth RG (2013b) Molecular pharmacology and ligand docking studies reveal a single amino acid difference between mouse and human serotonin 5-HT2A receptors that impacts behavioral translation of novel 4-phenyl-2-dimethylaminotetralin ligands. J Pharmacol Exp Ther 347:705–716

Canal CE, Felsing DE, Liu Y, Zhu W, Wood JT, Perry CK, Vemula R, Booth RG (2015) An orally active phenylaminotetralin-chemotype serotonin 5-HT7 and 5-HT1A receptor partial agonist that corrects motor stereotypy in mouse models. ACS Chem Neurosci 6:1259–1270

Canton H, Verriele L, Millan MJ (1994) Competitive antagonism of serotonin (5-HT)2C and 5-HT2A receptor-mediated phosphoinositide (PI) turnover by clozapine in the rat: a comparison to other antipsychotics. Neurosci Lett 181:65–68

Carbonaro TM, Eshleman AJ, Forster MJ, Cheng K, Rice KC, Gatch MB (2015) The role of 5-HT2A, 5-HT 2C and mGlu2 receptors in the behavioral effects of tryptamine hallucinogens *N,N*-dimethyltryptamine and *N,N*-diisopropyltryptamine in rats and mice. Psychopharmacology 232:275–284

Carhart-Harris RL, Erritzoe D, Williams T, Stone JM, Reed LJ, Colasanti A, Tyacke RJ, Leech R, Malizia AL, Murphy K, Hobden P, Evans J, Feilding A, Wise RG, Nutt DJ (2012) Neural correlates of the psychedelic state as determined by fMRI studies with psilocybin. Proc Natl Acad Sci 109:2138–2143

Carhart-Harris RL, Leech R, Hellyer PJ, Shanahan M, Feilding A, Tagliazucchi E, Chialvo DR, Nutt D (2014) The entropic brain: a theory of conscious states informed by neuroimaging research with psychedelic drugs. Front Hum Neurosci 8:20

Carhart-Harris RL, Muthukumaraswamy S, Roseman L, Kaelen M, Droog W, Murphy K, Tagliazucchi E, Schenberg EE, Nest T, Orban C, Leech R, Williams LT, Williams TM, Bolstridge M, Sessa B, McGonigle J, Sereno MI, Nichols D, Hellyer PJ, Hobden P, Evans J, Singh KD, Wise RG, Curran HV, Feilding A, Nutt DJ (2016) Neural correlates of the LSD experience revealed by multimodal neuroimaging. Proc Natl Acad Sci U S A 113:4853–4858

Carhart-Harris RL, Roseman L, Bolstridge M, Demetriou L, Pannekoek JN, Wall MB, Tanner M, Kaelen M, McGonigle J, Murphy K, Leech R, Curran HV, Nutt DJ (2017) Psilocybin for treatment-resistant depression: fMRI-measured brain mechanisms. Sci Rep 7:13187

Ceci C, Proietti Onori M, Macri S, Laviola G (2015) Interaction between the endocannabinoid and serotonergic system in the exhibition of head twitch response in four mouse strains. Neurotox Res 27:275–283

Chambers JJ, Nichols DE (2002) A homology-based model of the human 5-HT2A receptor derived from an in silico activated G-protein coupled receptor. J Comput Aided Mol Des 16:511–520

Cheng Y, Prusoff WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50% inhibition (I50) of an enzymatic reaction. Biochem Pharmacol 22:3099–3108

Choudhary MS, Sachs N, Uluer A, Glennon RA, Westkaemper RB, Roth BL (1995) Differential ergoline and ergopeptine binding to 5-hydroxytryptamine2A receptors: ergolines require an aromatic residue at position 340 for high affinity binding. Mol Pharmacol 47:450–457

Clark LD, Bliss EL (1957) Psychopharmacological studies of lysergic acid diethylamide (LSD-25) intoxication; effects of premedication with BOL-128 (2-bromo-d-lysergic acid diethylamide), mescaline, atropine, amobarbital, and chlorpromazine. AMA Arch Neurol Psychiatry 78:653–655

Cordova-Sintjago T, Villa N, Fang L, Booth RG (2014) Aromatic interactions impact ligand binding and function at serotonin 5-HT2C G protein-coupled receptors: receptor homology modeling, ligand docking, and molecular dynamics results validated by experimental studies. Mol Phys 112:398–407

Corne SJ, Pickering RW (1967) A possible correlation between drug-induced hallucinations in man and a behavioural response in mice. Psychopharmacologia 11:65–78

Cozzi NV, Daley PF (2016) Receptor binding profiles and quantitative structure-affinity relationships of some 5-substituted-*N,N*-diallyltryptamines. Bioorg Med Chem Lett 26:959–964

Cussac D, Boutet-Robinet E, Ailhaud MC, Newman-Tancredi A, Martel JC, Danty N, Rauly-Lestienne I (2008) Agonist-directed trafficking of signalling at serotonin 5-HT2A, 5-HT2B and 5-HT2C-VSV receptors mediated Gq/11 activation and calcium mobilisation in CHO cells. Eur J Pharmacol 594:32–38

Dakic V, Minardi Nascimento J, Costa Sartore R, Maciel RM, de Araujo DB, Ribeiro S, Martins-de-Souza D, Rehen SK (2017) Short term changes in the proteome of human cerebral organoids induced by 5-MeO-DMT. Sci Rep 7:12863

Darmani NA (1998) Cocaine and selective monoamine uptake blockers (sertraline, nisoxetine, and GBR 12935) prevent the d-fenfluramine-induced head-twitch response in mice. Pharmacol Biochem Behav 60:83–90

Darmani NA (2001) Cannabinoids of diverse structure inhibit two DOI-induced 5-HT (2A) receptor-mediated behaviors in mice. Pharmacol Biochem Behav 68:311–317

Darmani NA, Pandya DK (2000) Involvement of other neurotransmitters in behaviors induced by the cannabinoid CB1 receptor antagonist SR 141716A in naive mice. J Neural Transm (Vienna) 107:931–945

Darmani NA, Reeves SL (1996) The mechanism by which the selective 5-HT1A receptor antagonist S-(-) UH 301 produces head-twitches in mice. Pharmacol Biochem Behav 55:1–10

Darmani NA, Mock OB, Towns LC, Gerdes CF (1994) The head-twitch response in the least shrew (Cryptotis parva) is a 5-HT2- and not a 5-HT1C-mediated phenomenon. Pharmacol Biochem Behav 48:383–396

Darmani NA, Janoyan JJ, Kumar N, Crim JL (2003) Behaviorally active doses of the CB1 receptor antagonist SR 141716A increase brain serotonin and dopamine levels and turnover. Pharmacol Biochem Behav 75:777–787

De Gregorio D, Posa L, Ochoa-Sanchez R, McLaughlin R, Maione S, Comai S, Gobbi G (2016) The hallucinogen d-lysergic diethylamide (LSD) decreases dopamine firing activity through 5-HT1A, D2 and TAAR1 receptors. Pharmacol Res 113:81–91

de Witte WE, Danhof M, van der Graaf PH, de Lange EC (2016) In vivo target residence time and kinetic selectivity: the association rate constant as determinant. Trends Pharmacol Sci 37:831–842

Delille HK, Becker JM, Burkhardt S, Bleher B, Terstappen GC, Schmidt M, Meyer AH, Unger L, Marek GJ, Mezler M (2012) Heterocomplex formation of 5-HT2A-mGlu2 and its relevance for cellular signaling cascades. Neuropharmacology 62:2184–2191

DeVree BT, Mahoney JP, Vélez-Ruiz GA, Rasmussen SGF, Kuszak AJ, Edwald E, Fung J-J, Manglik A, Masureel M, Du Y, Matt RA, Pardon E, Steyaert J, Kobilka BK, Sunahara RK (2016) Allosteric coupling from G protein to the agonist-binding pocket in GPCRs. Nature 535:182

Di Matteo V, Di Giovanni G, Di Mascio M, Esposito E (2000) Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin (2C) receptors. Brain Res 865:85–90

Dittrich A (1998) The standardized psychometric assessment of altered states of consciousness (ASCs) in humans. Pharmacopsychiatry 31(Suppl 2):80–84

Domenech T, Beleta J, Palacios JM (1997) Characterization of human serotonin 1D and 1B receptors using [3H]-GR-125743, a novel radiolabelled serotonin 5HT1D/1B receptor antagonist. Naunyn Schmiedeberg's Arch Pharmacol 356:328–334

Done CJ, Sharp T (1992) Evidence that 5-HT2 receptor activation decreases noradrenaline release in rat hippocampus in vivo. Br J Pharmacol 107:240–245

Du C, Collins W, Cantley W, Sood D, Kaplan DL (2017) Tutorials for electrophysiological recordings in neuronal tissue engineering. ACS Biomater Sci Eng 3:2235–2246

Dursun SM, Handley SL (1993) The effects of alpha 2-adrenoceptor antagonists on the inhibition of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced head shakes by 5-HT1A receptor agonists in the mouse. Br J Pharmacol 109:1046–1052

Egan CT, Herrick-Davis K, Miller K, Glennon RA, Teitler M (1998a) Agonist activity of LSD and lisuride at cloned 5HT2A and 5HT2C receptors. Psychopharmacology 136:409–414

Egan CT, Herrick-Davis K, Teitler M (1998b) Creation of a constitutively activated state of the 5-hydroxytryptamine2A receptor by site-directed mutagenesis: inverse agonist activity of antipsychotic drugs. J Pharmacol Exp Ther 286:85–90

Egan C, Grinde E, Dupre A, Roth BL, Hake M, Teitler M, Herrick-Davis K (2000) Agonist high and low affinity state ratios predict drug intrinsic activity and a revised ternary complex mechanism at serotonin 5-HT(2A) and 5-HT(2C) receptors. Synapse 35:144–150

Egashira N, Mishima K, Uchida T, Hasebe N, Nagai H, Mizuki A, Iwasaki K, Ishii H, Nishimura R, Shoyama Y, Fujiwara M (2004) Anandamide inhibits the DOI-induced head-twitch response in mice. Psychopharmacology 171:382–389

Egashira N, Shirakawa A, Okuno R, Mishima K, Iwasaki K, Oishi R, Fujiwara M (2011) Role of endocannabinoid and glutamatergic systems in DOI-induced head-twitch response in mice. Pharmacol Biochem Behav 99:52–58

Fantegrossi WE, Harrington AW, Eckler JR, Arshad S, Rabin RA, Winter JC, Coop A, Rice KC, Woods JH (2005) Hallucinogen-like actions of 2,5-dimethoxy-4-(n)-propylthiophenethylamine (2C-T-7) in mice and rats. Psychopharmacology 181:496–503

Fantegrossi WE, Harrington AW, Kiessel CL, Eckler JR, Rabin RA, Winter JC, Coop A, Rice KC, Woods JH (2006) Hallucinogen-like actions of 5-methoxy-*N,N*-diisopropyltryptamine in mice and rats. Pharmacol Biochem Behav 83:122–129

Fantegrossi WE, Reissig CJ, Katz EB, Yarosh HL, Rice KC, Winter JC (2008) Hallucinogen-like effects of *N,N*-dipropyltryptamine (DPT): possible mediation by serotonin 5-HT1A and 5-HT2A receptors in rodents. Pharmacol Biochem Behav 88:358–365

Fantegrossi WE, Simoneau J, Cohen MS, Zimmerman SM, Henson CM, Rice KC, Woods JH (2010) Interaction of 5-HT2A and 5-HT2C receptors in R(-)-2,5-dimethoxy-4-iodoamphetamine-elicited head twitch behavior in mice. J Pharmacol Exp Ther 335:728–734

Felder CC, Kanterman RY, Ma AL, Axelrod J (1990) Serotonin stimulates phospholipase A2 and the release of arachidonic acid in hippocampal neurons by a type 2 serotonin receptor that is independent of inositolphospholipid hydrolysis. Proc Natl Acad Sci U S A 87:2187–2191

Fiorella D, Rabin RA, Winter JC (1995) The role of the 5-HT2A and 5-HT2C receptors in the stimulus effects of hallucinogenic drugs. I: antagonist correlation analysis. Psychopharmacology 121:347–356

Fontanilla D, Johannessen M, Hajipour AR, Cozzi NV, Jackson MB, Ruoho AE (2009) The hallucinogen *N,N*-dimethyltryptamine (DMT) is an endogenous sigma-1 receptor regulator. Science 323:934–937

Freedman DX, Giarman NJ (1962) LSD-25 and the status and level of brain serotonin. Ann N Y Acad Sci 96:98–107

Freud S, Byck R (1975) Cocaine papers. Stonehill, New York

Garcia EE, Smith RL, Sanders-Bush E (2007) Role of G(q) protein in behavioral effects of the hallucinogenic drug 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane. Neuropharmacology 52:1671–1677

Gatch MB, Rutledge MA, Carbonaro T, Forster MJ (2009) Comparison of the discriminative stimulus effects of dimethyltryptamine with different classes of psychoactive compounds in rats. Psychopharmacology 204:715–724

Gatch MB, Dolan SB, Forster MJ (2017) Locomotor and discriminative stimulus effects of four novel hallucinogens in rodents. Behav Pharmacol 28:375–385

Gellman RL, Aghajanian GK (1994) Serotonin2 receptor-mediated excitation of interneurons in piriform cortex: antagonism by atypical antipsychotic drugs. Neuroscience 58:515–525

Ghoneim OM, Legere JA, Golbraikh A, Tropsha A, Booth RG (2006) Novel ligands for the human histamine H1 receptor: synthesis, pharmacology, and comparative molecular field analysis studies of 2-dimethylamino-5-(6)-phenyl-1,2,3,4-tetrahydronaphthalenes. Bioorg Med Chem 14:6640–6658

Giarman NJ, Freedman DX (1965) Biochemical aspects of the actions of psychotomimetic drugs. Pharmacol Rev 17:1–25

Glennon RA (1991) Discriminative stimulus properties of hallucinogens and related designer drugs. NIDA Res Monogr 116:25–44

Glennon RA (1992) Animal models for assessing hallucinogenic agents. In: Boulton AA, Baker GB, Wu PH (eds) Animal models of drug addiction. Humana Press, Totowa, pp 345–381

Glennon RA (2017) The 2014 Philip S. Portoghese Medicinal Chemistry Lectureship: the "Phenylalkylaminome" with a focus on selected drugs of abuse. J Med Chem 60:2605–2628

Glennon RA, Young R (2011) Drug discrimination: applications to medicinal chemistry and drug studies. Wiley, Hoboken

Glennon RA, Young R, Rosecrans JA (1983) Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT2 antagonists. Eur J Pharmacol 91:189–196

Glennon RA, Titeler M, McKenney JD (1984) Evidence for 5-HT2 involvement in the mechanism of action of hallucinogenic agents. Life Sci 35:2505–2511

Glennon RA, Higgs R, Young R, Issa H (1992) Further studies on *N*-methyl-1 (3,4-methylenedioxyphenyl)-2-aminopropane as a discriminative stimulus: antagonism by 5-hydroxytryptamine3 antagonists. Pharmacol Biochem Behav 43:1099–1106

Gobert A, Millan MJ (1999) Serotonin (5-HT)2A receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. Neuropharmacology 38:315–317

Gonzalez-Maeso J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R, Lira A, Bradley-Moore M, Ge Y, Zhou Q, Sealfon SC, Gingrich JA (2007) Hallucinogens recruit specific cortical 5-HT (2A) receptor-mediated signaling pathways to affect behavior. Neuron 53:439–452

Gonzalez-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF, Zhou M, Okawa Y, Callado LF, Milligan G, Gingrich JA, Filizola M, Meana JJ, Sealfon SC (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. Nature 452:93–97

Goodwin GM, Green AR (1985) A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT1 and 5-HT2 receptors. Br J Pharmacol 84:743–753

Gresch PJ, Barrett RJ, Sanders-Bush E, Smith RL (2007) 5-Hydroxytryptamine (serotonin)2A receptors in rat anterior cingulate cortex mediate the discriminative stimulus properties of d-lysergic acid diethylamide. J Pharmacol Exp Ther 320:662–669

Griebel G, Pichat P, Boulay D, Naimoli V, Potestio L, Featherstone R, Sahni S, Defex H, Desvignes C, Slowinski F, Vige X, Bergis OE, Sher R, Kosley R, Kongsamut S, Black MD, Varty GB (2016) The mGluR2 positive allosteric modulator, SAR218645, improves memory and attention deficits in translational models of cognitive symptoms associated with schizophrenia. Sci Rep 6:35320

Griffiths RR, Johnson MW, Carducci MA, Umbricht A, Richards WA, Richards BD, Cosimano MP, Klinedinst MA (2016) Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: a randomized double-blind trial. J Psychopharmacol 30:1181–1197

Grundmann M, Kostenis E (2017) Temporal bias: time-encoded dynamic GPCR signaling. Trends Pharmacol Sci 38:1110–1124

Gudelsky GA, Yamamoto BK, Nash JF (1994) Potentiation of 3,4-methylenedioxymethamphetamine-induced dopamine release and serotonin neurotoxicity by 5-HT2 receptor agonists. Eur J Pharmacol 264:325–330

Halberstadt AL (2017) Pharmacology and toxicology of *N*-benzylphenethylamine ("NBOMe") hallucinogens. Curr Top Behav Neurosci 32:283–311

Halberstadt AL, Geyer MA (2013) Characterization of the head-twitch response induced by hallucinogens in mice: detection of the behavior based on the dynamics of head movement. Psychopharmacology 227:727–739

Halberstadt AL, Geyer MA (2014) Effects of the hallucinogen 2,5-dimethoxy-4-iodophenethylamine (2C-I) and superpotent *N*-benzyl derivatives on the head twitch response. Neuropharmacology 77:200–207

Halberstadt AL, Geyer MA (2017) Effect of hallucinogens on unconditioned behavior. Curr Top Behav Neurosci. https://doi.org/10.1007/7854_2016_466

Halberstadt AL, Koedood L, Powell SB, Geyer MA (2011) Differential contributions of serotonin receptors to the behavioral effects of indoleamine hallucinogens in mice. J Psychopharmacol 25:1548–1561

Hartman JL, Northup JK (1996) Functional reconstitution in situ of 5-hydroxytryptamine2c (5HT2c) receptors with alphaq and inverse agonism of 5HT2c receptor antagonists. J Biol Chem 271:22591–22597

Herrick-Davis K, Grinde E, Teitler M (2000) Inverse agonist activity of atypical antipsychotic drugs at human 5-hydroxytryptamine2C receptors. J Pharmacol Exp Ther 295:226–232

Hide I, Kato T, Yamawaki S (1989) In vivo determination of 5-hydroxytryptamine receptor-stimulated phosphoinositide turnover in rat brain. J Neurochem 53:556–560

Hoffmann C, Leitz MR, Oberdorf-Maass S, Lohse MJ, Klotz K-N (2004) Comparative pharmacology of human β-adrenergic receptor subtypes – characterization of stably transfected receptors in CHO cells. Naunyn Schmiedeberg's Arch Pharmacol 369:151–159

Hua T, Vemuri K, Pu M, Qu L, Han GW, Wu Y, Zhao S, Shui W, Li S, Korde A, Laprairie RB, Stahl EL, Ho JH, Zvonok N, Zhou H, Kufareva I, Wu B, Zhao Q, Hanson MA, Bohn LM, Makriyannis A, Stevens RC, Liu ZJ (2016) Crystal structure of the human cannabinoid receptor CB1. Cell 167(750–762):e14

Hua T, Vemuri K, Nikas SP, Laprairie RB, Wu Y, Qu L, Pu M, Korde A, Jiang S, Ho JH, Han GW, Ding K, Li X, Liu H, Hanson MA, Zhao S, Bohn LM, Makriyannis A, Stevens RC, Liu ZJ (2017) Crystal structures of agonist-bound human cannabinoid receptor CB1. Nature 547:468–471

Isbell H, Logan CR (1957) Studies on the diethylamide of lysergic acid (LSD-25). II. Effects of chlorpromazine, azacyclonol, and reserpine on the intensity of the LSD-reaction. A M A Arch Neurol Psychiatry 77:350–358

Isberg V, Balle T, Sander T, Jorgensen FS, Gloriam DE (2011) G protein- and agonist-bound serotonin 5-HT2A receptor model activated by steered molecular dynamics simulations. J Chem Inf Model 51:315–325

Iversen L, Gibbons S, Treble R, Setola V, Huang X-P, Roth BL (2013) Neurochemical profiles of some novel psychoactive substances. Eur J Pharmacol 700:147–151

Jensen AA, McCorvy JD, Leth-Petersen S, Bundgaard C, Liebscher G, Kenakin TP, Brauner-Osborne H, Kehler J, Kristensen JL (2017) Detailed characterization of the in vitro pharmacological and pharmacokinetic properties of *N*-(2-hydroxybenzyl)-2,5-dimethoxy-4-cyanophenylethylamine (25CN-NBOH), a highly selective and brain-penetrant 5-HT2A receptor agonist. J Pharmacol Exp Ther 361:441–453

Johansen A, Hansen HD, Svarer C, Lehel S, Leth-Petersen S, Kristensen JL, Gillings N, Knudsen GM (2017) The importance of small polar radiometabolites in molecular neuroimaging: a PET study with [(11)C]Cimbi-36 labeled in two positions. J Cereb Blood Flow Metab. https://doi.org/10.1177/0271678X17746179

Jope RS, Song L, Powers R (1994) [3H]PtdIns hydrolysis in postmortem human brain membranes is mediated by the G-proteins Gq/11 and phospholipase C-beta. Biochem J 304(Pt 2):655–659

Kadamur G, Ross EM (2013) Mammalian phospholipase C. Annu Rev Physiol 75:127–154
Karaki S, Becamel C, Murat S, Mannoury la Cour C, Millan MJ, Prezeau L, Bockaert J, Marin P, Vandermoere F (2014) Quantitative phosphoproteomics unravels biased phosphorylation of serotonin 2A receptor at Ser280 by hallucinogenic versus nonhallucinogenic agonists. Mol Cell Proteomics 13:1273–1285
Katritch V, Cherezov V, Stevens RC (2013) Structure-function of the G protein-coupled receptor superfamily. Annu Rev Pharmacol Toxicol 53:531–556
Kehne JH, Baron BM, Carr AA, Chaney SF, Elands J, Feldman DJ, Frank RA, van Giersbergen PL, McCloskey TC, Johnson MP, McCarty DR, Poirot M, Senyah Y, Siegel BW, Widmaier C (1996) Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100,907 as a potent 5-HT2A antagonist with a favorable CNS safety profile. J Pharmacol Exp Ther 277:968–981
Keller DL, Umbreit WW (1956) Permanent alteration of behavior in mice by chemical and psychological means. Science 124:723–724
Kenakin T (2016) Measurement of receptor signaling bias. Curr Protoc Pharmacol 74:2–15
Kennedy RT (2013) Emerging trends in in vivo neurochemical monitoring by microdialysis. Curr Opin Chem Biol 17:860–867
Kometer M, Vollenweider FX (2016) Serotonergic hallucinogen-induced visual perceptual alterations. Curr Top Behav Neurosci. https://doi.org/10.1007/7854_2016_461
Kometer M, Schmidt A, Bachmann R, Studerus E, Seifritz E, Vollenweider FX (2012) Psilocybin biases facial recognition, goal-directed behavior, and mood state toward positive relative to negative emotions through different serotonergic subreceptors. Biol Psychiatry 72:898–906
Kometer M, Schmidt A, Jancke L, Vollenweider FX (2013) Activation of serotonin 2A receptors underlies the psilocybin-induced effects on alpha oscillations, N170 visual-evoked potentials, and visual hallucinations. J Neurosci 33:10544–10551
Kraehenmann R, Pokorny D, Vollenweider L, Preller KH, Pokorny T, Seifritz E, Vollenweider FX (2017) Dreamlike effects of LSD on waking imagery in humans depend on serotonin 2A receptor activation. Psychopharmacology 234:2031–2046
Kroeze WK, Hufeisen SJ, Popadak BA, Renock SM, Steinberg S, Ernsberger P, Jayathilake K, Meltzer HY, Roth BL (2003) H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. Neuropsychopharmacology 28:519–526
Kurrasch-Orbaugh DM, Parrish JC, Watts VJ, Nichols DE (2003a) A complex signaling cascade links the serotonin2A receptor to phospholipase A2 activation: the involvement of MAP kinases. J Neurochem 86:980–991
Kurrasch-Orbaugh DM, Watts VJ, Barker EL, Nichols DE (2003b) Serotonin 5-hydroxytryptamine 2A receptor-coupled phospholipase C and phospholipase A2 signaling pathways have different receptor reserves. J Pharmacol Exp Ther 304:229–237
Lee MY, Chiang CC, Chiu HY, Chan MH, Chen HH (2014) *N*-acetylcysteine modulates hallucinogenic 5-HT(2A) receptor agonist-mediated responses: behavioral, molecular, and electrophysiological studies. Neuropharmacology 81:215–223
Leysen JE, Eens A, Gommeren W, Van Gompel P, Wynants J, Janssen PA (1987) Non-serotonergic [3H]ketanserin binding sites in striatal membranes are associated with a dopac release system on dopaminergic nerve endings. Eur J Pharmacol 134:373–375
Leysen JE, Janssen PM, Schotte A, Luyten WH, Megens AA (1993) Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of 5HT2 receptors. Psychopharmacology 112:S40–S54
Li JX, Rice KC, France CP (2007) Behavioral effects of dipropyltryptamine in rats: evidence for 5-HT1A and 5-HT2A agonist activity. Behav Pharmacol 18:283–288
Li JX, Rice KC, France CP (2009a) Discriminative stimulus effects of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane in rhesus monkeys: antagonism and apparent pA2 analyses. J Pharmacol Exp Ther 328:976–981
Li JX, Unzeitig A, Javors MA, Rice KC, Koek W, France CP (2009b) Discriminative stimulus effects of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), ketanserin, and (R)-(+)-{alpha}-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemetha nol (MDL100907) in rats. J Pharmacol Exp Ther 331:671–679

Liechti ME (2017) Modern clinical research on LSD. Neuropsychopharmacology 42:2114–2127
Liu Y, Canal CE, Cordova-Sintjago TC, Zhu W, Booth RG (2017) Mutagenesis analysis reveals distinct amino acids of the human serotonin 5-HT2C receptor underlying the pharmacology of distinct ligands. ACS Chem Neurosci 8:28–39
Lopez-Gimenez JF, Vilaro MT, Palacios JM, Mengod G (1998) [3H]MDL 100,907 labels 5-HT2A serotonin receptors selectively in primate brain. Neuropharmacology 37:1147–1158
Lopez-Gimenez JF, Vilaro MT, Palacios JM, Mengod G (2013) Multiple conformations of 5-HT2A and 5-HT 2C receptors in rat brain: an autoradiographic study with [125I](+/−)DOI. Exp Brain Res 230:395–406
Luparini MR, Garrone B, Pazzagli M, Pinza M, Pepeu G (2004) A cortical GABA-5HT interaction in the mechanism of action of the antidepressant trazodone. Prog Neuro-Psychopharmacol Biol Psychiatry 28:1117–1127
Marona-Lewicka D, Thisted RA, Nichols DE (2005) Distinct temporal phases in the behavioral pharmacology of LSD: dopamine D2 receptor-mediated effects in the rat and implications for psychosis. Psychopharmacology 180:427–435
Martin DA, Nichols CD (2017) The effects of hallucinogens on gene expression. Curr Top Behav Neurosci. https://doi.org/10.1007/7854_2017_479
Martin-Ruiz R, Puig MV, Celada P, Shapiro DA, Roth BL, Mengod G, Artigas F (2001) Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. J Neurosci 21:9856–9866
May JA, Sharif NA, Chen HH, Liao JC, Kelly CR, Glennon RA, Young R, Li JX, Rice KC, France CP (2009) Pharmacological properties and discriminative stimulus effects of a novel and selective 5-HT2 receptor agonist AL-38022A [(S)-2-(8,9-dihydro-7H-pyrano[2,3-g]indazol-1-yl)-1-methylethylamine]. Pharmacol Biochem Behav 91:307–314
Mayberg HS, Brannan SK, Mahurin RK, Jerabek PA, Brickman JS, Tekell JL, Silva JA, McGinnis S, Glass TG, Martin CC, Fox PT (1997) Cingulate function in depression: a potential predictor of treatment response. Neuroreport 8:1057–1061
McCreary AC, Filip M, Cunningham KA (2003) Discriminative stimulus properties of (+/−)-fenfluramine: the role of 5-HT2 receptor subtypes. Behav Neurosci 117:212–221
McGrew L, Chang MS, Sanders-Bush E (2002) Phospholipase D activation by endogenous 5-hydroxytryptamine 2C receptors is mediated by Galpha13 and pertussis toxin-insensitive Gbetagamma subunits. Mol Pharmacol 62:1339–1343
McKenna DJ, Saavedra JM (1987) Autoradiography of LSD and 2,5-dimethoxyphenylisopropylamine psychotomimetics demonstrates regional, specific cross-displacement in the rat brain. Eur J Pharmacol 142:313–315
McKenna DJ, Mathis CA, Shulgin AT, Sargent T 3rd, Saavedra JM (1987) Autoradiographic localization of binding sites for 125I-DOI, a new psychotomimetic radioligand, in the rat brain. Eur J Pharmacol 137:289–290
McKinney M, Raddatz R (2006) Practical aspects of radioligand binding. Curr Protoc Pharmacol 1:1.3. https://doi.org/10.1002/0471141755.ph0103s33
Milligan G, Kostenis E (2006) Heterotrimeric G-proteins: a short history. Br J Pharmacol 147(Suppl 1):S46–S55
Montgomery T, Buon C, Eibauer S, Guiry PJ, Keenan AK, McBean GJ (2007) Comparative potencies of 3,4-methylenedioxymethamphetamine (MDMA) analogues as inhibitors of [(3)H]noradrenaline and [(3)H]5-HT transport in mammalian cell lines. Br J Pharmacol 152:1121–1130
Moreau JJ (1973) Hashish and mental illness. Raven Press, New York
Moreno JL, Holloway T, Albizu L, Sealfon SC, Gonzalez-Maeso J (2011) Metabotropic glutamate mGlu2 receptor is necessary for the pharmacological and behavioral effects induced by hallucinogenic 5-HT2A receptor agonists. Neurosci Lett 493:76–79
Moutkine I, Quentin E, Guiard BP, Maroteaux L, Doly S (2017) Heterodimers of serotonin receptor subtypes 2 are driven by 5-HT2C protomers. J Biol Chem 292:6352–6368

Moya PR, Berg KA, Gutierrez-Hernandez MA, Saez-Briones P, Reyes-Parada M, Cassels BK, Clarke WP (2007) Functional selectivity of hallucinogenic phenethylamine and phenylisopropylamine derivatives at human 5-hydroxytryptamine (5-HT)2A and 5-HT2C receptors. J Pharmacol Exp Ther 321:1054–1061

Mueller F, Lenz C, Dolder PC, Harder S, Schmid Y, Lang UE, Liechti ME, Borgwardt S (2017) Acute effects of LSD on amygdala activity during processing of fearful stimuli in healthy subjects. Transl Psychiatry 7:e1084

Muschamp JW, Regina MJ, Hull EM, Winter JC, Rabin RA (2004) Lysergic acid diethylamide and [-]-2,5-dimethoxy-4-methylamphetamine increase extracellular glutamate in rat prefrontal cortex. Brain Res 1023:134–140

Nair SG, Gudelsky GA (2004) Activation of 5-HT2 receptors enhances the release of acetylcholine in the prefrontal cortex and hippocampus of the rat. Synapse 53:202–207

Navailles S, De Deurwaerdere P, Spampinato U (2006) Clozapine and haloperidol differentially alter the constitutive activity of central serotonin2C receptors in vivo. Biol Psychiatry 59:568–575

Nichols DE (2016) Psychedelics. Pharmacol Rev 68:264–355

Nichols DE (2017) Chemistry and structure-activity relationships of psychedelics. Curr Top Behav Neurosci. https://doi.org/10.1007/7854_2017_475

Nichols DE, Frescas SP, Chemel BR, Rehder KS, Zhong D, Lewin AH (2008) High specific activity tritium-labeled *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine (INBMeO): a high-affinity 5-HT2A receptor-selective agonist radioligand. Bioorg Med Chem 16:6116–6123

Nichols DE, Sassano MF, Halberstadt AL, Klein LM, Brandt SD, Elliott SP, Fiedler WJ (2015) *N*-Benzyl-5-methoxytryptamines as potent serotonin 5-HT2 receptor family agonists and comparison with a series of phenethylamine analogues. ACS Chem Neurosci 6:1165–1175

Nichols DE, Johnson MW, Nichols CD (2017) Psychedelics as medicines: an emerging new paradigm. Clin Pharmacol Ther 101:209–219

Pahnke WN, Richards WA (1966) Implications of LSD and experimental mysticism. J Relig Health 5:175–208

Pahnke WN, Kurland AA, Unger S, Savage C, Grof S (1970) The experimental use of psychedelic (LSD) psychotherapy. JAMA 212:1856–1863

Palfreyman MG, Schmidt CJ, Sorensen SM, Dudley MW, Kehne JH, Moser P, Gittos MW, Carr AA (1993) Electrophysiological, biochemical and behavioral evidence for 5-HT2 and 5-HT3 mediated control of dopaminergic function. Psychopharmacology 112:S60–S67

Palhano-Fontes F, Andrade KC, Tofoli LF, Santos AC, Crippa JA, Hallak JE, Ribeiro S, de Araujo DB (2015) The psychedelic state induced by ayahuasca modulates the activity and connectivity of the default mode network. PLoS One 10:e0118143

Parrish JC, Nichols DE (2006) Serotonin 5-HT(2A) receptor activation induces 2-arachidonoylglycerol release through a phospholipase c-dependent mechanism. J Neurochem 99:1164–1175

Parrish JC, Braden MR, Gundy E, Nichols DE (2005) Differential phospholipase C activation by phenylalkylamine serotonin 5-HT 2A receptor agonists. J Neurochem 95:1575–1584

Pauwels PJ, Van Gompel P, Leysen JE (1993) Activity of serotonin (5-HT) receptor agonists, partial agonists and antagonists at cloned human 5-HT1A receptors that are negatively coupled to adenylate cyclase in permanently transfected HeLa cells. Biochem Pharmacol 45:375–383

Pazos A, Probst A, Palacios JM (1987) Serotonin receptors in the human brain – IV. Autoradiographic mapping of serotonin-2 receptors. Neuroscience 21:123–139

Pehek EA, Hernan AE (2015) Stimulation of glutamate receptors in the ventral tegmental area is necessary for serotonin-2 receptor-induced increases in mesocortical dopamine release. Neuroscience 290:159–164

Pehek EA, McFarlane HG, Maguschak K, Price B, Pluto CP (2001) M100,907, a selective 5-HT2A antagonist, attenuates dopamine release in the rat medial prefrontal cortex. Brain Res 888:51–59

Pehek EA, Nocjar C, Roth BL, Byrd TA, Mabrouk OS (2006) Evidence for the preferential involvement of 5-HT2A serotonin receptors in stress- and drug-induced dopamine release in the rat medial prefrontal cortex. Neuropsychopharmacology 31:265–277

Peng Y, McCorvy JD, Harpsøe K, Lansu K, Yuan S, Popov P, Qu L, Pu M, Che T, Nikolajsen LF, Huang X-P, Wu Y, Shen L, Bjørn-Yoshimoto WE, Ding K, Wacker D, Han GW, Cheng J, Katritch V, Jensen AA, Hanson MA, Zhao S, Gloriam DE, Roth BL, Stevens RC, Liu Z-J (2018) 5-HT2C receptor structures reveal the structural basis of GPCR polypharmacology. Cell 172(4):719–730.e14

Perez-Aguilar JM, Shan J, LeVine MV, Khelashvili G, Weinstein H (2014) A functional selectivity mechanism at the serotonin-2A GPCR involves ligand-dependent conformations of intracellular loop 2. J Am Chem Soc 136:16044–16054

Petri G, Expert P, Turkheimer F, Carhart-Harris R, Nutt D, Hellyer PJ, Vaccarino F (2014) Homological scaffolds of brain functional networks. J R Soc Interface 11:20140873

Pokorny T, Preller KH, Kraehenmann R, Vollenweider FX (2016) Modulatory effect of the 5-HT1A agonist buspirone and the mixed non-hallucinogenic 5-HT1A/2A agonist ergotamine on psilocybin-induced psychedelic experience. Eur Neuropsychopharmacol 26:756–766

Preller KH, Vollenweider FX (2016) Phenomenology, structure, and dynamic of psychedelic states. Curr Top Behav Neurosci. https://doi.org/10.1007/7854_2016_459

Preller KH, Herdener M, Pokorny T, Planzer A, Kraehenmann R, Stampfli P, Liechti ME, Seifritz E, Vollenweider FX (2017) The fabric of meaning and subjective effects in LSD-induced states depend on serotonin 2A receptor activation. Curr Biol 27:451–457

Puig MV, Watakabe A, Ushimaru M, Yamamori T, Kawaguchi Y (2010) Serotonin modulates fast-spiking interneuron and synchronous activity in the rat prefrontal cortex through 5-HT1A and 5-HT2A receptors. J Neurosci 30:2211–2222

Quednow BB, Geyer MA, Halberstadt AL (2010) Serotonin and schizophrenia. In: Muller CP, Jacobs B (eds) Handbook of the behavioral neurobiology of serotonin. Academic Press, London, pp 585–620

Quednow BB, Kometer M, Geyer MA, Vollenweider FX (2012) Psilocybin-induced deficits in automatic and controlled inhibition are attenuated by ketanserin in healthy human volunteers. Neuropsychopharmacology 37:630–640

Rabin RA, Regina M, Doat M, Winter JC (2002) 5-HT2A receptor-stimulated phosphoinositide hydrolysis in the stimulus effects of hallucinogens. Pharmacol Biochem Behav 72:29–37

Ranganathan A, Rodríguez D, Carlsson J (2017) Structure-based discovery of GPCR ligands from crystal structures and homology models. Springer, Berlin, Heidelberg, pp 1–35

Raote I, Bhattacharyya S, Panicker MM (2013) Functional selectivity in serotonin receptor 2A (5-HT2A) endocytosis, recycling, and phosphorylation. Mol Pharmacol 83:42–50

Rauser L, Savage JE, Meltzer HY, Roth BL (2001) Inverse agonist actions of typical and atypical antipsychotic drugs at the human 5-hydroxytryptamine(2C) receptor. J Pharmacol Exp Ther 299:83–89

Reissig CJ, Eckler JR, Rabin RA, Winter JC (2005) The 5-HT1A receptor and the stimulus effects of LSD in the rat. Psychopharmacology 182:197–204

Richards N, Chapman LF, Goodell H, Wolff HG (1958) LSD-like delirium following ingestion of a small amount of its brom analog (BOL-148). Ann Intern Med 48:1078–1082

Richelson E, Souder T (2000) Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. Life Sci 68:29–39

Rickli A, Kopf S, Hoener MC, Liechti ME (2015a) Pharmacological profile of novel psychoactive benzofurans. Br J Pharmacol 172:3412–3425

Rickli A, Luethi D, Reinisch J, Buchy D, Hoener MC, Liechti ME (2015b) Receptor interaction profiles of novel *N*-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). Neuropharmacology 99:546–553

Rickli A, Moning OD, Hoener MC, Liechti ME (2016) Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. Eur Neuropsychopharmacol 26:1327–1337

Riva-Posse P, Choi KS, Holtzheimer PE, Crowell AL, Garlow SJ, Rajendra JK, McIntyre CC, Gross RE, Mayberg HS (2017) A connectomic approach for subcallosal cingulate deep brain stimulation surgery: prospective targeting in treatment-resistant depression. Mol Psychiatry. https://doi.org/10.1038/mp.2017.59

Ross S, Bossis A, Guss J, Agin-Liebes G, Malone T, Cohen B, Mennenga SE, Belser A, Kalliontzi K, Babb J, Su Z, Corby P, Schmidt BL (2016) Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial. J Psychopharmacol 30:1165–1180

Roth BL, Choudhary MS, Khan N, Uluer AZ (1997) High-affinity agonist binding is not sufficient for agonist efficacy at 5-hydroxytryptamine2A receptors: evidence in favor of a modified ternary complex model. J Pharmacol Exp Ther 280:576–583

Roth BL, Lopez E, Patel S, Kroeze WK (2000) The multiplicity of serotonin receptors: uselessly diverse molecules or an embarrassment of riches? Neuroscientist 6:252–262

Saleh N, Ibrahim P, Saladino G, Gervasio FL, Clark T (2017) An efficient metadynamics-based protocol to model the binding affinity and the transition state ensemble of G-protein-coupled receptor ligands. J Chem Inf Model 57:1210–1217

Sanders-Bush E, Burris KD, Knoth K (1988) Lysergic acid diethylamide and 2,5-dimethoxy-4-methylamphetamine are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis. J Pharmacol Exp Ther 246:924–928

Santana N, Artigas F (2017) Expression of serotonin2c receptors in pyramidal and GABAergic neurons of rat prefrontal cortex: a comparison with striatum. Cereb Cortex 27:3125–3139

Santana N, Mengod G, Artigas F (2013) Expression of alpha(1)-adrenergic receptors in rat prefrontal cortex: cellular co-localization with 5-HT(2A) receptors. Int J Neuropsychopharmacol 16:1139–1151

Schmid CL, Bohn LM (2010) Serotonin, but not N-methyltryptamines, activates the serotonin 2A receptor via a ss-arrestin2/Src/Akt signaling complex in vivo. J Neurosci 30:13513–13524

Schmid CL, Raehal KM, Bohn LM (2008) Agonist-directed signaling of the serotonin 2A receptor depends on beta-arrestin-2 interactions in vivo. Proc Natl Acad Sci U S A 105:1079–1084

Schmid CL, Streicher JM, Meltzer HY, Bohn LM (2014) Clozapine acts as an agonist at serotonin 2A receptors to counter MK-801-induced behaviors through a betaarrestin2-independent activation of Akt. Neuropsychopharmacology 39:1902–1913

Schreiber R, Brocco M, Millan MJ (1994) Blockade of the discriminative stimulus effects of DOI by MDL 100,907 and the 'atypical' antipsychotics, clozapine and risperidone. Eur J Pharmacol 264:99–102

Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S, Millan MJ (1995) (1-(2,5-dimethoxy-4 iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT) 2A receptors: modulation by novel 5-HT2A/2C antagonists, D1 antagonists and 5-HT1A agonists. J Pharmacol Exp Ther 273:101–112

Scruggs JL, Schmidt D, Deutch AY (2003) The hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) increases cortical extracellular glutamate levels in rats. Neurosci Lett 346:137–140

Shulgin A, Shulgin A (1991) Pihkal: a chemical love story. Transform Press, Berkeley

Shulgin A, Shulgin A (1997) Tihkal: the continuation. Transform Press, Berkeley

Simmler LD, Buchy D, Chaboz S, Hoener MC, Liechti ME (2016) In vitro characterization of psychoactive substances at rat, mouse, and human trace amine-associated receptor 1. J Pharmacol Exp Ther 357:134–144

Snyder SH, Faillace L, Hollister L (1967) 2,5-Dimethoxy-4-methyl-amphetamine (STP): a new hallucinogenic drug. Science 158:669–670

Spindle MS, Thomas MP (2014) Activation of 5-HT2A receptors by TCB-2 induces recurrent oscillatory burst discharge in layer 5 pyramidal neurons of the mPFC in vitro. Physiol Rep 2: e12003. https://doi.org/10.14814/phy2.12003

Strassman R (2001) DMT: the spirit molecule: a doctor's revolutionary research into the biology of near-death and mystical experiences. Park Street Press, Rochester

Studerus E, Gamma A, Kometer M, Vollenweider FX (2012) Prediction of psilocybin response in healthy volunteers. PLoS One 7:e30800

Sykes DA, Dowling MR, Charlton SJ (2010) Measuring receptor target coverage: a radioligand competition binding protocol for assessing the association and dissociation rates of unlabeled compounds. Curr Protoc Pharmacol 9:14. https://doi.org/10.1002/0471141755.ph0914s50

Tagliazucchi E, Carhart-Harris R, Leech R, Nutt D, Chialvo DR (2014) Enhanced repertoire of brain dynamical states during the psychedelic experience. Hum Brain Mapp 35:5442–5456

Tagliazucchi E, Roseman L, Kaelen M, Orban C, Muthukumaraswamy SD, Murphy K, Laufs H, Leech R, McGonigle J, Crossley N, Bullmore E, Williams T, Bolstridge M, Feilding A, Nutt DJ, Carhart-Harris R (2016) Increased global functional connectivity correlates with LSD-induced ego dissolution. Curr Biol 26:1043–1050

Thal DM, Sun B, Feng D, Nawaratne V, Leach K, Felder CC, Bures MG, Evans DA, Weis WI, Bachhawat P, Kobilka TS, Sexton PM, Kobilka BK, Christopoulos A (2016) Crystal structures of the M1 and M4 muscarinic acetylcholine receptors. Nature 531:335–340

Thomsen WJ, Grottick AJ, Menzaghi F, Reyes-Saldana H, Espitia S, Yuskin D, Whelan K, Martin M, Morgan M, Chen W, Al-Shamma H, Smith B, Chalmers D, Behan D (2008) Lorcaserin, a novel selective human 5-hydroxytryptamine2C agonist: in vitro and in vivo pharmacological characterization. J Pharmacol Exp Ther 325:577–587

Tian MK, Schmidt EF, Lambe EK (2016) Serotonergic suppression of mouse prefrontal circuits implicated in task attention. eNeuro 3. https://doi.org/10.1523/ENEURO.0269-16.2016

Valle M, Maqueda AE, Rabella M, Rodriguez-Pujadas A, Antonijoan RM, Romero S, Alonso JF, Mananas MA, Barker S, Friedlander P, Feilding A, Riba J (2016) Inhibition of alpha oscillations through serotonin-2A receptor activation underlies the visual effects of ayahuasca in humans. Eur Neuropsychopharmacol 26:1161–1175

Verhoeff NP, Visser WH, Ferrari MD, Saxena PR, van Royen EA (1993) Dopamine D2-receptor imaging with 123I-iodobenzamide SPECT in migraine patients abusing ergotamine: does ergotamine cross the blood brain barrier? Cephalalgia 13:325–329

Viol A, Palhano-Fontes F, Onias H, de Araujo DB, Viswanathan GM (2017) Shannon entropy of brain functional complex networks under the influence of the psychedelic Ayahuasca. Sci Rep 7:7388

Vollenweider FX (2001) Brain mechanisms of hallucinogens and entactogens. Dialogues Clin Neurosci 3:265–279

Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Babler A, Vogel H, Hell D (1998) Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. Neuroreport 9:3897–3902

Wacker D, Fenalti G, Brown MA, Katritch V, Abagyan R, Cherezov V, Stevens RC (2010) Conserved binding mode of human β2 adrenergic receptor inverse agonists and antagonist revealed by X-ray crystallography. J Am Chem Soc 132:11443–11445

Wacker D, Wang C, Katritch V, Han GW, Huang XP, Vardy E, McCorvy JD, Jiang Y, Chu M, Siu FY, Liu W, Xu HE, Cherezov V, Roth BL, Stevens RC (2013) Structural features for functional selectivity at serotonin receptors. Science 340:615–619

Wacker D, Wang S, McCorvy JD, Betz RM, Venkatakrishnan AJ, Levit A, Lansu K, Schools ZL, Che T, Nichols DE, Shoichet BK, Dror RO, Roth BL (2017) Crystal structure of an LSD-bound human serotonin receptor. Cell 168:377–389.e12

Walker SJ, Brown HA, Molecular and Cellular Biology CUINYUSA (2004) Measurement of G protein-coupled receptor-stimulated phospholipase D activity in intact cells. Methods Mol Biol 237:89–97

Wang C, Jiang Y, Ma J, Wu H, Wacker D, Katritch V, Han GW, Liu W, Huang XP, Vardy E, McCorvy JD, Gao X, Zhou XE, Melcher K, Zhang C, Bai F, Yang H, Yang L, Jiang H, Roth BL, Cherezov V, Stevens RC, Xu HE (2013) Structural basis for molecular recognition at serotonin receptors. Science 340:610–614

Wang S, Wacker D, Levit A, Che T, Betz RM, McCorvy JD, Venkatakrishnan AJ, Huang XP, Dror RO, Shoichet BK, Roth BL (2017) D4 dopamine receptor high-resolution structures enable the discovery of selective agonists. Science 358:381–386

Weber ET, Andrade R (2010) Htr2a gene and 5-HT(2A) receptor expression in the cerebral cortex studied using genetically modified mice. Front Neurosci 4:36. https://doi.org/10.3389/fnins.2010.00036

Wenthur CJ, Lindsley CW (2013) Classics in chemical neuroscience: clozapine. ACS Chem Neurosci 4:1018–1025

Willins DL, Meltzer HY (1997) Direct injection of 5-HT2A receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. J Pharmacol Exp Ther 282:699–706

Willins DL, Deutch AY, Roth BL (1997) Serotonin 5-HT2A receptors are expressed on pyramidal cells and interneurons in the rat cortex. Synapse 27:79–82

Winter JC (2009) Hallucinogens as discriminative stimuli in animals: LSD, phenethylamines, and tryptamines. Psychopharmacology 203:251–263

Winter CA, Flataker L (1956) Effects of lysergic acid diethylamide upon performance of trained rats. Proc Soc Exp Biol Med 92:285–289

Winter JC, Rice KC, Amorosi DJ, Rabin RA (2007) Psilocybin-induced stimulus control in the rat. Pharmacol Biochem Behav 87:472–480

Wright IK, Garratt JC, Marsden CA (1990) Effects of a selective 5-HT2 agonist, DOI, on 5-HT neuronal firing in the dorsal raphe nucleus and 5-HT release and metabolism in the frontal cortex. Br J Pharmacol 99:221–222

Xia Z, Gray JA, Compton-Toth BA, Roth BL (2003) A direct interaction of PSD-95 with 5-HT2A serotonin receptors regulates receptor trafficking and signal transduction. J Biol Chem 278:21901–21908

Yang KC, Stepanov V, Martinsson S, Ettrup A, Takano A, Knudsen GM, Halldin C, Farde L, Finnema SJ (2017) Fenfluramine reduces [11C]Cimbi-36 binding to the 5-HT2A receptor in the nonhuman primate brain. Int J Neuropsychopharmacol 20:683–691

Zhang ZW, Arsenault D (2005) Gain modulation by serotonin in pyramidal neurones of the rat prefrontal cortex. J Physiol 566:379–394

Zhelyazkova-Savova M, Giovannini MG, Pepeu G (1997) Increase of cortical acetylcholine release after systemic administration of chlorophenylpiperazine in the rat: an in vivo microdialysis study. Neurosci Lett 236:151–154

Zhelyazkova-Savova M, Giovannini MG, Pepeu G (1999) Systemic chlorophenylpiperazine increases acetylcholine release from rat hippocampus-implication of 5-HT2C receptors. Pharmacol Res 40:165–170

Phencyclidine-Based New Psychoactive Substances

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Abstract

The serendipitous discovery of phencyclidine (PCP) in 1956 sets the stage for significant research efforts that resulted in a plethora of analogs and derivatives designed to explore the biological effects of this class. PCP soon became the prototypical dissociative agent that eventually sneaked through the doors of clinical laboratories and became an established street drug. Estimations suggest that around 14 PCP analogs were identified as "street drugs" in the period between the 1960s and 1990s. Fast forward to the 2000s, and largely facilitated by advancements in electronic forms of communication made possible through the Internet, a variety of new PCP analogs began to attract the attention of communities interested in the collaborative exploration of these substances. Traditionally, as was the case with the first-generation analogs identified in previous decades, the substances explored represented compounds already known in the scientific literature. As the decade of the noughties unfolded, a number of new PCP-derived substances appeared on the scene, which included some analogs that have not been previously recorded in the published literature. The aim of this chapter is to present a brief introductory overview of substances that have materialized as PCP-derived new psychoactive substances (NPS) in recent years and their known pharmacology. Since *N*-methyl-D-aspartate receptor (NMDAR) antagonism is implicated in mediating the subjective and mind-altering effects of many dissociative drugs, additional data are included from other analogs not presently identified as NPS.

Keywords

Clinical · Designer drugs · Dissociatives · Forensic · NMDA receptor · NPS · Pharmacology · Phencyclidine · Toxicology

Acronyms of the Discussed New Psychoactive Substances (NPS)

2-MeO-PCMo	4-[1-(2-Methoxyphenyl)cyclohexyl]morpholine
3,4-MD-PCE	1-(1,3-Benzodioxol-5-yl)-*N*-ethylcyclohexanamine
3,4-MD-PCMo	4-[1-(1,3-Benzodioxol-5-yl)cyclohexyl]morpholine

3,4-MD-PCPr	1-(1,3-Benzodioxol-5-yl)-*N*-propylcyclohexanamine
3,4-MD-PCPy	1-[1-(1,3-Benzodioxol-5-yl)cyclohexyl]pyrrolidine
3-HO-PCE	3-[1-(Ethylamino)cyclohexyl]phenol
3-HO-PCMe	1-3-Hydroxyphenyl)-*N*-methylcyclohexanamine
3-HO-PCP	3-[1-(Piperidin-1-yl)cyclohexyl]phenol
3-HO-PCPr	1-3-Hydroxyphenyl)-*N*-propylcyclohexanamine
3-Me-4-F-PCP	1-[1-(4-Fluoro-3-methylphenyl)cyclohexyl]piperidine
3-Me-4-MeO-PCP	1-[1-(4-Methoxy-3-methylphenyl)cyclohexyl]piperidine
3-MeO-PCE	*N*-Ethyl-1-(3-methoxyphenyl)cyclohexan-1-amine
3-MeO-PCMe	1-(3-Methoxyphenyl)-*N*-methylcyclohexan-1-amine
3-MeO-PCMMo	4-{[1-(3-Methoxyphenyl)cyclohexyl]methyl}morpholine
3-MeO-PCMo	4-[1-(3-Methoxyphenyl)cyclohexyl]morpholine
3-MeO-PCP	1-[1-(3-Methoxyphenyl)cyclohexyl]piperidine
3-MeO-PCPr	1-(3-Methoxyphenyl)-*N*-propylcyclohexan-1-amine
3-MeO-PCPy	1-[1-(3-Methoxyphenyl)cyclohexyl]pyrrolidine
3-Me-PCMo	4-[1-(3-Methylphenyl)cyclohexyl]morpholine
3-Me-PCPMe	*N*-Methyl-1-(3-methylphenyl)cyclohexanamine
3-Me-PCPy	1-[1-(3-Methylphenyl)cyclohexyl]pyrrolidine
4′-Me-PCP	1-(4-Methyl-1-phenylcyclohexyl)piperidine
4-MeO-PCMo	4-[1-(4-Methoxyphenyl)cyclohexyl]morpholine
4-MeO-PCP	1-[1-(4-Methoxyphenyl)cyclohexyl]piperidine (methoxydine)
4-MeO-PCPy	1-[1-(4-Methoxyphenyl)cyclohexyl]pyrrolidine
4-Me-PCP	1-[1-(4-Methylphenyl)cyclohexyl]piperidine
AB-FUBINACA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1*H*-indazole-3-carboxamide
BnCP	1-(1-Benzylcyclohexyl)piperidine
DXM	Dextromethorphan
MDPV	1-(2*H*-1,3-Benzodioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one
MK-801	(+)-10,11-Dihydro-5*H*-5,10-epiminodibenzo[*a*,*d*][7]annulene (dizocilpine)
MXE	2-(Ethylamino)-2-(3-methoxyphenyl)cyclohexan-1-one (methoxetamine)
PCE	*N*-Ethyl-1-phenylcyclohexan-1-amine (eticyclidine)
PCEEA	*N*-(2-Ethoxyethyl)-1-phenylcyclohexan-1-amine
PCHOEA	2-[(1-Phenylcyclohexyl)amino]ethan-1-ol
PCiP	1-Phenyl-*N*-(propan-2-yl)cyclohexan-1-amine
PCMEA	*N*-(2-Methoxyethyl)-1-phenylcyclohexan-1-amine
PCMo	4-(1-Phenylcyclohexyl)morpholine
PCMPA	*N*-(3-Methoxypropyl)-1-phenylcyclohexan-1-amine
PCP	1-(1-Phenylcyclohexyl)piperidine (phencyclidine)
PCPr	1-Phenyl-*N*-propylcyclohexan-1-amine
PCPy	1-(1-Phenylcyclohexyl)pyrrolidine (rolicyclidine)
TCP	1-[1-(Thiophen-2-yl)cyclohexyl]piperidine (tenocyclidine)
TCPy	1-[1-(Thiophen-2-yl)cyclohexyl]pyrrolidine
U-49900	3,4-Dichloro-*N*-[(1*R*,2*R*)-2-(diethylamino)cyclohexyl]-*N*-methylbenzamide

1 Introduction

Following the serendipitous discovery of phencyclidine (PCP, Fig. 1) in 1956, an avalanche of research efforts emerged over the following decades, which resulted in the exploration of many interesting analogs. These agents were developed in the pursuit of novel therapeutic agents as well as to probe the biological effects in humans and other animals and to understand the function and distribution of PCP binding sites in a variety of in vitro and in vivo test systems (e.g., Petersen and Stillman 1978; Domino 1981; Kamenka and Geneste 1983; Clouet 1986; Domino and Kamenka 1988).

One of the terms used to describe the effects of PCP (and ketamine) is "dissociative anesthetic," which was termed by Toni Domino, the wife of PCP and ketamine researcher Edward F. Domino, in an effort to name this unique class of agents (Domino 2010). The term has since been shortened to "dissociative" to account for the wide variety of clinical uses and effects of these agents. The subjective effects of dissociative drugs are complex and highly dose dependent. For example, low doses typically induce a state of intoxication subjectively comparable to ethanol, which has also been consistent with drug discrimination assays in rodents (Hundt et al. 1998; Krystal et al. 1998; Lodge and Mercier 2015). Psychostimulant

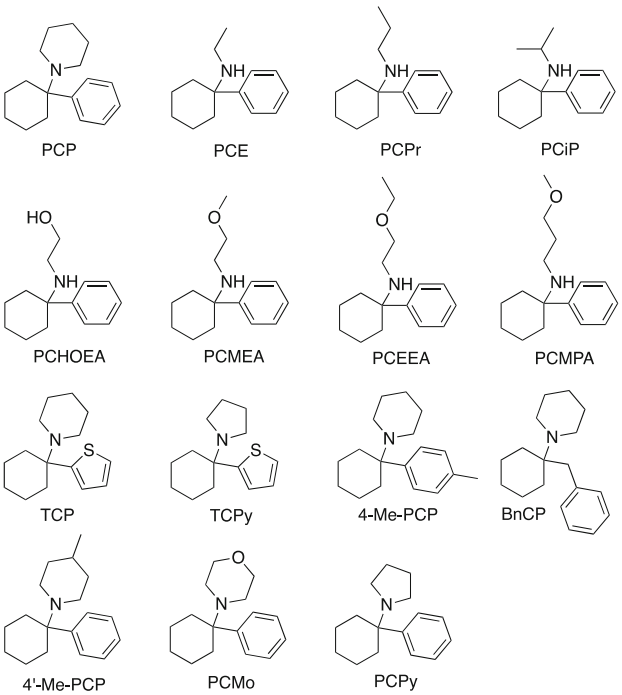

Fig. 1 Phencyclidine (PCP) and examples of first-generation analogs that appeared on the streets between the 1960s and 1990s (Morris and Wallach 2014)

properties are often reported by humans and seen in other animals especially with lower doses (Lodge and Mercier 2015). Other common subjective effects of dissociatives include euphoria, tactile, visual and auditory hallucinations, altered thought patterns, paresthesia, depersonalization, and derealization (Morris and Wallach 2014; Steinpreis 1996). Higher doses in humans and other animals often lead to full dissociation from the sensory environment, anesthesia, catalepsy, and motor impairment. The available data obtained from experiments with human subjects and ethnographic studies showed that subjective effects can vary substantially depending on set and setting in which the drug experience occurs (Pollard et al. 1965; Feldman et al. 1979).

N-Methyl-D-aspartate (NMDA) receptor antagonism is implicated in mediating, at least in part, the subjective and mind-altering effects of many compounds classified as dissociatives (Morris and Wallach 2014; Lodge and Mercier 2015). This is also supported by the fact that structurally diverse antagonists of multiple binding sites on the NMDAR are known to induce such effects. Similarly, this is consistent with the finding that antibodies against NMDAR, as seen in anti-NMDA receptor encephalitis, also induce dissociative effects in humans (Gable et al. 2009). NMDAR is a ligand-(glutamate and glycine as co-agonists) and voltage-gated ion channel, which plays central roles in important biological mechanisms including synaptic signaling and plasticity, cell survival, information processing, memory and learning (Ogden and Traynelis 2011; Hardingham and Bading 2003). NMDAR also has many roles in the periphery including the immune system (Boldyrev et al. 2005). NMDAR has been considered as a potential target of intervention for various disease states including pain, epilepsy, neurodegenerative diseases, tinnitus, and numerous psychiatric disorders (Cull-Candy et al. 2001; Javitt 2007; Paoletti et al. 2013; Traynelis et al. 2010). Currently, it appears that PCP and related arylcyclohexylamines and many related dissociative compounds like MK-801 and diphenidine act as use-dependent (uncompetitive) antagonists of NMDAR by binding inside the channel pore to a site called the PCP binding site. This leads to a blockade of ion conductance through the channel (Kemp and McKernan 2002; Lodge and Mercier 2015). The crystal structure of the heterotetrameric (GluN1/GluN2B) NMDAR was recently solved by two groups, though the PCP binding site within the channel pore was poorly resolved (Lee et al. 2014; Karakas and Furukawa 2014). More recently a cryo-EM structure of a hetereotrimeric NMDAR was published, which has provided information on MK-801 binding inside the channel pore (Lü et al. 2017). Docking studies using homology have been performed and may provide some insight into the PCP binding site (Tikhonova et al. 2004; El M'Barki and Elhallaqui 2017).

The nonmedical use of PCP-derived substances by humans is not a recent phenomenon. It has been estimated that in the period between the 1960s and 1990s, around 14 PCP analogs associated with nonmedical use and availability on the street market have been identified, such as PCE, PCPr, PCiP, PCHOEA, PCMEA, PCEEA, PCMPA, TCP, TCPy, 4-Me-PCP, BnCP, 4′-Me-PCP, PCMo, and PCPy (Fig. 1). Nevertheless, it appeared that only some of these first-generation analogs, such as TCP, PCE, and PCPy, were widely used (Morris and Wallach 2014). Fast forward to the 2000s, and with the advent of the Internet, the exchange of information and the extent of distribution of psychoactive drugs changed

dramatically. Early dissociative drugs sold online included ketamine and DXM. Then around 2008 what appears to be one of the first research chemical dissociatives, 4-MeO-PCP (Fig. 2a), began to appear for sale online. A number of analogs of PCP marketed as research chemicals soon followed which continues to this day. The majority of substances that have so far appeared over the last few decades originated from "legitimate" drug discovery research. However, some other PCP-based analogs represented true inventions made outside the traditional scientific setting. Perhaps one of the most striking aspects related to these particular examples is the ability for dissociative drug *aficionados* to foster information exchange, development of ideas, and collaborations involving online discussion boards that yield novel substances (Morris and Wallach 2014). Some user forums are more sophisticated than others in

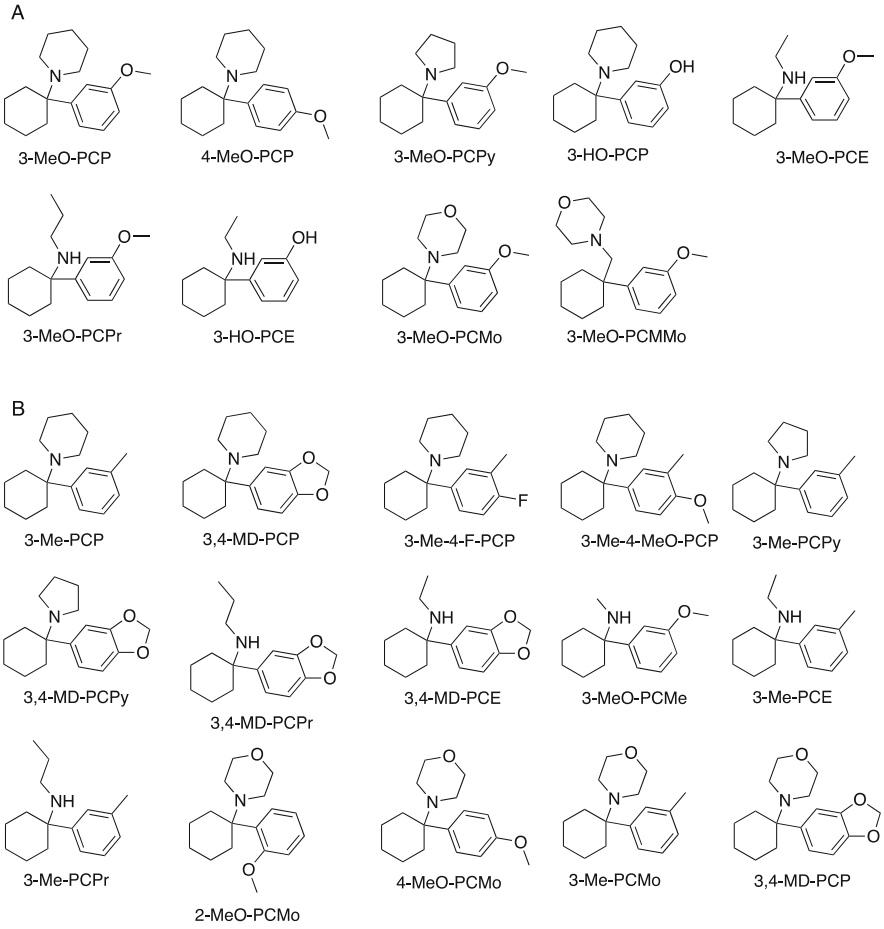

Fig. 2 (**a**) Phencyclidine analogs that appeared as a new psychoactive substance in recent years. (**b**) Representative examples of PCP analogs that have undergone biological testing but which have not yet appeared on the NPS market (Wallach 2014; Colestock et al. 2018)

terms of the extent of exchanging scientific knowledge. Information exchange about the effects and circumstances of drug use can also provide opportunities for exploring harm reduction advice shared between users, at least between those who engage in online technology as recently discussed within the context of dissociative NPS use (Hearne and Van Hout 2016). Examples also exist where users of dissociative substances make clear references to self-medication and treatment (Morris and Wallach 2014), adding to speculations and discussions around the mechanisms of action associated with some of these substances and how these might affect the users' conditions (Coppola and Mondola 2013). Attempting to review the knowledge collected on the large number of PCP-based substances developed over the last 40 years is beyond the scope of this chapter, but it is meant to provide an introduction to some of the dissociative new psychoactive substances (NPS) that have emerged on the market in recent years.

2 PCP

Phencyclidine (PCP) is a potent analgesic and dissociative drug that elicits its effect when administered through oral or parenteral routes. Following its discovery, PCP emerged as a potent anesthetic agent for use in humans under the brand name Serynl®; however, significant untoward effects were soon discovered. These effects included severe cognitive and sensory impairment/deprivation, agitation, and aggressive and bizarre behavior, particularly at higher dosage levels, that led to its discontinuation (Greifenstein et al. 1958; Luby et al. 1959; Meyer et al. 1959; Domino 1964). PCP also was used briefly as a veterinary general anesthetic (Morris and Wallach 2014).

PCP has been investigated extensively in humans and other animals. Doses used in human volunteers depended on the routes of administration and the intent of the study, which impacted on the effects observed. It is also worth acknowledging that early PCP administration studies found that schizophrenic patients were much more sensitive to the drug (Domino 1964). Both PCP and ketamine have been used to evaluate whether these substances elicit certain aspects of psychosis including positive and negative symptoms of schizophrenia in humans and other animals. Based on this research, both PCP and ketamine have been used in research as a model for psychosis (Javitt and Zukin 1991; Murray 2002; Frohlich and Van Horn 2014; Lodge and Mercier 2015; Cadinu et al. 2017). As summarized by Pradhan (1984), minimal intoxicating doses of PCP were reported to be up to 1 mg (intravenously, iv), whereas subanesthetic doses used in several clinical studies were 0.1 mg/kg. Anesthetic doses used in surgical patients were around 0.25 mg/kg iv, and it was noticed that an increase to higher doses (0.50 and 1.0 mg/kg, iv) produced agitation, purposeless movements, and seizures. Oral doses have been administered to up to 30 mg, and inhalation studies carried out in humans suggested that retained doses above 0.225 mg/kg resulted in collapse, prostration, and incapacitation (Pradhan 1984). Even when given at subanesthetic doses (iv, 0.1 mg/kg), significant sensory and cognitive effects were observed, such as alterations in body image, estrangement and isolation, negativism, hostility, drowsiness and apathy, hypnogenic states,

feelings of inebriation, and repetitive motor behavior. Other commonly observed features included rotatory nystagmus, ataxic and slapping gait, and nausea (Luby et al. 1959). More intense reactions were reported by Meyer et al. (1959) who described that 13 out of 80 subjects who received "large" doses (iv) developed a "confusional psychosis" of 12–72 h duration "characterized by feelings of unreality, depression, anxiety, and delusional and illusional experience." The spectrum of effects reported by recreational PCP users has also been reported (Burns and Lerner 1976; Siegel 1978). In addition to the already mentioned literature sources mentioned above, additional historical information on PCP might also be obtained from Lodge and Mercier (2015) and Morris and Wallach (2014). In order to contextualize the emergence of PCP-derived NPS, a brief overview of some of the representative studies and relevant pharmacological data on PCP are presented here.

2.1 Pharmacokinetics

PCP is a weak tertiary amine base with an experimentally derived pKa of 8.5 (Baselt 2011). PCP is lipophilic and Kamenka and Geneste (1981) gave the experimentally derived logP as 5.1. Based on investigations with human volunteers (Cook et al. 1982), plasma binding was determined to be about 65%, and oral bioavailability was estimated at 72%. The apparent terminal phase half-life ($T_{1/2}$) was reported around 21 ± 3 h (harmonic mean 17 h, range 7–46 h) (Cook et al. 1982). In cases of overdose, however, the $T_{1/2}$ has been reported to be up to 4 days (Done et al. 1978; Jackson 1989). Consistent with its high lipophilicity, PCP has been shown to accumulate in adipose and brain tissue (Bey and Patel 2007). Brain concentrations typically exceed blood concentrations (up to twice the amount) as determined in fatal over cases (Cravey et al. 1979). Likewise, PCP has been found to have a fairly large volume of distribution of 6.2 ± 0.3 L/kg (Cook et al. 1982) or 5.3–7.5 L/kg [mean, ~6 L/kg] (Barceloux 2012). A number of studies have investigated the metabolism of PCP (e.g., Wall et al. 1981; Aniline and Pitts 1982; Hallstrom et al. 1983; Cho et al. 1983; Holsztynska and Domino 1983, 1985). Mono-hydroxylations of the cyclohexyl and piperidine ring have been found to be major metabolites detected in urine of humans and animals (Aniline and Pitts 1982; Holsztynska and Domino 1983). PCP is also metabolized through phase II metabolic pathways (including glucuronide and sulfate conjugates) via conjugation of its phase I metabolites (Holsztynska and Domino 1985). Induction of liver enzymes has been found to impact on the metabolism of PCP (Kammerer et al. 1984). Excretion of PCP from human male subjects was found to be largely through urine (72.8 ± 4.0% of dose) with minor amounts found in feces (4.7 ± 0.9%) and perspiration (after iv injection of 1 mg) (Cook et al. 1982).

2.2 Pharmacodynamic Effects In Vitro

PCP has high affinity for the PCP binding site of NMDAR, originally named for the high affinity seen for PCP (Table 1). PCP acts as an uncompetitive use-dependent

inhibitor of NMDAR by blocking the open channel pore (Lodge and Mercier 2015; Wallach 2014). Since NMDAR is a hetereotetrameric receptor, composed of multiple possible subunits, subtype specificity of PCP and related channel blockers for NMDAR types has been reported, and the extent to which this influences the pharmacology of these compounds has been investigated to some extent (reviewed in Lodge and Mercier 2015). In addition, PCP and other high-affinity NMDAR antagonists like MK-801 appear to have long dissociation off-rates and thus become trapped in the channel, leading to longer duration of channel block (Lodge and Mercier 2015). Dissociative effects seen in humans typically correlate well with affinities toward the PCP site of NMDAR and PCP-like discrimination in animal models (Lodge and Mercier 2015). It should be noted that evidence suggests that channel blockers with faster off-rates and modest affinity, like memantine, may be better tolerated therapeutically than compounds with high affinity and slow dissociation off-rates (Wallach 2014; Lipton 2006).

In addition to NMDAR, PCP also has been reported to have affinities at a number of other central nervous system receptor sites with varying potencies. For example, PCP was also found to act as a monoamine reuptake transporter blocker (Aniline and Pitts 1982). Likewise, PCP has been reported to have affinity at the human reuptake transporter of serotonin (SERT) but not dopamine (DAT) or norephinephrine (NET) expressed in HEK293 cells (Table 1) (Roth et al. 2013, 2018). However other studies have shown activities with these transporters. For example, Giros et al. (1992) reported inhibition of [^{3}H]DA reuptake with affinities (K_i = 677 and 430 nM) for rat and human DAT expressed in mouse fibroblast Ltk- cells. Likewise, PCP was found to inhibit monoamine uptake of norepinephrine (NE) (IC_{50} = 520 nM), serotonin (5-HT) (IC_{50} = 800 nM), and dopamine (DA) (IC_{50} = 730 nM) in crude rat brain synaptosomal preparations. PCP had potency similar to d-amphetamine and methylphenidate for catecholamines and was 8 and 34 times more potent than d-amphetamine and methylphenidate in inhibiting [^{3}H]5-HT uptake, respectively (Smith et al. 1977). PCP has shown modest affinity for sigma receptors labeled through several techniques (Largent et al. 1986), and more recently, had high affinity for sigma-2 (K_i = 136 nM) but not sigma-1 (IC_{50} > 10,000 nM) (Roth et al. 2013). PCP has also been found to act as an antagonist at acetylcholine receptors and to inhibit acetylcholinesterase (Aniline and Pitts 1982). [^{3}H]Morphine displacement by PCP was reported using rat brain preparations albeit with a weak K_i value of 11,000 nM. Weak potency was also observed against other opioid radioligands including [^{3}H]leucine-enkephalin (K_i = 73 μM), [^{3}H]ethylketocyclazocine (K_i = 4,100 nM) and [^{3}H]-SKF-10,047 (K_i = 710 nM) (Itzhak et al. 1981a). In comparison, a K_i value of 26,000 nM for PCP against [^{3}H]morphine in rat brain homogenates was reported by Kamenka et al. (1982). Reports of affinity for dopamine D_2 receptors have appeared (Kapur and Seeman 2002; Seeman et al. 2005) although attempts by others to replicate these findings were unsuccessful under the conditions used (Roth et al. 2018; Jordan et al. 2006). PCP was also reported to have modest affinity at 5-HT_2 receptors (K_i = 5,000 nM) by Kapur and Seeman (2002) but not (IC_{50} > 10,000 nM) in another study (Roth et al. 2018).

Table 1 Representative in vitro binding data of NPS arylcyclohexylamines related to NMDAR, NET, DAT and SERT

Compound	NMDAR	NET	DAT	SERT
PCP	$K_i = 22.1$ nM Colestock et al. (2018) $K_i = 57.9$ nM Wallach (2014) $K_i = 59$ nM Roth et al. (2013) $K_i = 250$ nM Vignon et al. (1982), Chaudieu et al. (1989), and Vignon et al. (1988) $IC_{50} = 27.3$ nM Mendelsohn et al. (1984) $IC_{50} = 90$ nM Quirion et al. (1981) $IC_{50} = 37$ nM Ponchant et al. (1990) $IC_{50} = 250$ nM ([^{3}H]PCP) $IC_{50} = 200$ nM ([^{3}H]TCP) Chaudieu et al. (1987) $IC_{50} = 21$ nM Itzhak (1988)	$IC_{50} > 10{,}000$ nM Roth et al. (2013)	$K_i = 726$ nM Vignon et al. (1988) $IC_{50} = 760$ nM Hamon et al. (1996) $IC_{50} > 10{,}000$ nM Roth et al. (2018) K_i = 430 nM (human) K_i = 677 (rat) Giros et al. (1992)	$K_i = 2{,}234$ nM Roth et al. (2013) IC_{50} = 3000 nM Hori et al. (1996)
PCMo	$K_i = 334.1$ nM Colestock et al. (2018)	$IC_{50} > 10{,}000$ nM Colestock et al. (2018)	$IC_{50} > 10{,}000$ Colestock et al. (2018)	$IC_{50} > 10{,}000$ Colestock et al. (2018)
3-HO-PCE	$IC_{50} = 23$ nM Reel et al. (1988)	–	–	–
3-HO-PCMe	$IC_{50} = 422$ nM Reel et al. (1988)	–	–	–
3-HO-PCP	$K_i = 30$ nM Kamenka et al. (1982), Vignon et al. (1982), Vignon et al. (1988), and Chaudieu et al. (1989) $IC_{50} = 7.4$ nM	$IC_{50} > 10{,}000$ nM Wallach (2014)	$K_i = 1{,}154$ nM Wallach (2014) $IC_{50} = 1{,}360$ nM Vignon et al. (1988)	$IC_{50} > 10{,}000$ Wallach (2014)

	Mendelsohn et al. (1984) $IC_{50} = 6.3$ nM Ponchant et al. (1990) $K_i = 7.4–17.9$ nM Suzuki et al. (1996) $IC_{50} = 30$ nM ([^{3}H]PCP) $IC_{50} = 45$ nM ([^{3}H]TCP) Chaudieu et al. (1987) $IC_{50} = 2$ nM Itzhak (1988)			
3-HO-PCPr	$IC_{50} = 39$ nM Reel et al. (1988)	–	–	–
3-Me-PCE	$K_i = 43.1$ nM Wallach (2014)	$K_i = 598$ nM Wallach (2014)	$IC_{50} > 10{,}000$ nM Wallach (2014)	$K_i = 117$ nM Wallach (2014)
3-Me-PCMe	$K_i = 134.8$ nM Wallach (2014)	$IC_{50} > 10{,}000$ nM Wallach (2014)	$K_i = 1{,}729$ nM Wallach (2014)	$K_i = 1{,}389$ nM Wallach (2014)
3-Me-PCMo	$K_i = 201.8$ nM Colestock et al. (2018)	$IC_{50} > 10{,}000$ nM Colestock et al. (2018)	$K_i = 306$ nM Colestock et al. (2018)	$K_i = 412$ nM Colestock et al. (2018)
3-Me-PCPr	$K_i = 53.5$ nM Wallach (2014)	$K_i = 264$ nM Wallach (2014)	$K_i = 113$ nM Wallach (2014)	$K_i = 853$ nM Wallach (2014)
2-MeO-PCMo	$K_i = 1{,}578$ nM Colestock et al. (2018)	$IC_{50} > 10{,}000$ nM Colestock et al. (2018)	$IC_{50} > 10{,}000$ nM Colestock et al. (2018)	$K_i = 507$ nM Colestock et al. (2018)
3-MeO-PCMo	$K_i = 252.9$ nM Colestock et al. (2018)	$IC_{50} > 10{,}000$ nM Colestock et al. (2018)	$IC_{50} > 10{,}000$ nM Colestock et al. (2018)	$K_i = 697$ nM Colestock et al. (2018)
3-MeO-PCE	$K_i = 30.4$ nM Wallach (2014) $K_i = 61$ nM	$K_i = 1{,}528$ nM Wallach (2014)	$K_i = 906$ nM Wallach (2014)	$K_i = 136$ nM Wallach (2014)

(continued)

Table 1 (continued)

Compound	NMDAR	NET	DAT	SERT
	Roth et al. (2013) 99 nM Reel et al. (1988)	$IC_{50} > 10{,}000$ nM Roth et al. (2013)	$K_i = 743$ nM Roth et al. (2018)	$K_i = 115$ nM Roth et al. (2013)
3-MeO-PCMe	$K_i = 145.1$ nM Wallach (2014) $IC_{50} = 384$ nM Reel et al. (1988)	$K_i = 3{,}238$ nM Wallach (2014)	$IC_{50} > 10{,}000$ nM Wallach (2014)	$K_i = 1{,}368$ nM Wallach (2014)
3-MeO-PCP	$K_i = 38.1$ nM Wallach (2014) $K_i = 20$ nM Roth et al. (2013) $IC_{50} = 90$ nM Vignon et al. (1982), Vignon et al. (1988) and Chaudieu et al. (1989)	$K_i = 1{,}808$ nM Wallach (2014) $IC_{50} > 10{,}000$ nM Roth et al. (2013)	$IC_{50} > 10{,}000$ nM Wallach (2014) $K_i = 743$ nM Roth et al. (2013) $IC_{50} > 10{,}000$ nM Roth et al. (2018) $IC_{50} = 490$ nM Vignon et al. (1988) and Chaudieu et al. (1989)	$K_i = 1{,}571$ nM Wallach (2014) $K_i = 216$ nM Roth et al. (2013)
3-MeO-PCPr	$K_i = 17.9$ nM Wallach (2014) 53% [^{3}H]PCP displacement at 100 nM Reel et al. (1988)	$K_i = 1{,}342$ nM Wallach (2014)	$K_i = 381$ nM Wallach (2014)	$K_i = 700$ nM Wallach (2014)
3-MeO-PCPy	$K_i = 22.3$ nM Wallach (2014)	$K_i = 96$ nM Wallach (2014)	$IC_{50} > 10{,}000$ nM Wallach (2014)	$K_i = 11$ nM Wallach (2014)
3,4-MD-PCE	$K_i = 35.5$ nM Wallach (2014)	$IC_{50} > 10{,}000$ nM Wallach (2014)	$K_i = 2{,}867$ nM Wallach (2014)	$K_i = 637$ nM Wallach (2014)
3,4-MD-PCMo	$K_i = 425.5$ nM Colestock et al. (2018)	$IC_{50} > 10{,}000$ nM Colestock et al. (2018)	$IC_{50} > 10{,}000$ nM Colestock et al. (2018)	$IC_{50} > 10{,}000$ nM Colestock et al. (2018)
3,4-MD-PCPr	$K_i = 24.9$ nM Wallach (2014)	$IC_{50} > 10{,}000$ nM Wallach (2014)	$K_i = 1{,}940$ nM Wallach (2014)	$IC_{50} > 10{,}000$ nM Wallach (2014)

4-MeO-PCP	K_i = 620 nM Wallach (2014) K_i = 404 nM Roth et al. (2013) K_i = 500 nM Vignon et al. (1988) K_i = 1,200 nM Vignon et al. (1982) and Kamenka and Geneste (1983)	K_i = 1,811 nM Wallach (2014) K_i = 713 nM Roth et al. (2013)	IC_{50} > 10,000 nM Roth et al. (2013) K_i = 3,890 nM Vignon et al. (1988)	K_i = 900.7 nM Wallach (2014) K_i = 844 nM Roth et al. (2013)
4-MeO-PCMo	K_i = 2,118 nM Colestock et al. (2018)	IC_{50} > 10,000 nM Colestock et al. (2018)	K_i = 399 nM Colestock et al. (2018)	IC_{50} > 10,000 nM Colestock et al. (2018)
Ketamine	K_i = 323.9 nM Wallach et al. (2016a) K_i = 659 nM Roth et al. (2013) K_i = 800 nM Quirion et al. (1981)	IC_{50} > 10,000 nM Roth et al. (2013) K_i = 66.8 μM Nishimura et al. (1998)	IC_{50} > 10,000 nM Roth et al. (2018) K_i = 62.9 μM Nishimura et al. (1998)	IC_{50} > 10,000 nM Roth et al. (2013) K_i = 161.7 μM Nishimura et al. (1998)

NMDAR N-methyl-D-aspartate receptor, *NET* norepinephrine transporter, *DAT* dopamine transporter, *SERT* serotonin transporter. Radioligands and tissue preparations used for NMDAR binding, [^{3}H]MK-801 (rat brain cortex): Roth et al. (2013), Colestock et al. (2018) (rat forebrain), Wallach et al. (2016a) and Wallach (2014) (rat forebrain). [^{3}H]PCP: Quirion et al. (1981) (rat olfactory bulb slices), Kamenka et al. (1982) (rat brain), Vignon et al. (1982) (rat brain minus cerebellum), Mendelsohn et al. (1984) (rat cortex), Reel et al. (1988) (rat cortex), Vignon et al. (1988) (rat striata), Chaudieu et al. (1987) (rat brain), Chaudieu et al. (1989) (rat brain minus brainstem and cerebellum). [^{3}H]TCP: Chaudieu et al. (1987) (rat brain), Ponchant et al. (1990) (rat brain). [^{3}H]3-HO-PCP: Itzhak (1988) (rat brain). Suzuki et al. (1996) (displacement of [^{3}H]3-HO-PCP in various rat brain regions). Radioligands and tissue preparations used for monoamine reuptake transporters, [^{3}H]nisoxetine (NET), [^{3}H]WIN35,428 (DAT), [^{3}H]citalopram (SERT) (human proteins in transfected HEK293 cells): Wallach (2014), Roth et al. (2013), Roth et al. (2018). [^{3}H]BCTP: Hamon et al. (1996) (rat brain), Vignon et al. 1988 (rat striatal homogenates). [^{3}H]DA, [^{3}H]NE, [^{3}H]5-HT (Human NET and DAT, SERT (rat) in transfected HEK293 cells): Nishimura et al. (1998). [^{3}H]DA (human and rat DAT transfected mouse fibroblast Ltk$^-$ cells): Giros et al. (1992). [^{3}H]paroxetine (rat brain): Hori et al. (1996)

2.3 Effects In Vivo

The behavioral effects of PCP are species and dose dependent. Generally, a biphasic dose response has been observed in which low doses cause excitatory effects and high doses lead to a sedative and cataleptic response (Chen 1981; Balster and Chait 1976; Lodge and Mercier 2015). PCP blocked the tonic hindlimb extension in the maximal electroshock seizure (MES) test in male swiss albino mice (ip, $ED_{50} = 3.1$ mg/kg) (Rogawski et al. 1989). NMDAR antagonism has been implicated in anticonvulsant effects of arylcyclohexylamines in the rodent MES test based on high correlation between potencies and PCP binding site affinities (Leander et al. 1988; Wallach 2014). PCP has been found to cause potent rotarod impairment in mice ($ED_{50} = 4$ mg/kg) (Kamenka et al. 1982) and has also shown motor impairment in the horizontal screen test in male mice ip, $ED_{50} = 1.9$ mg/kg (Rogawski et al. 1989; Leander et al. 1988). For many arylcyclohexylamines, strong correlations have been observed between PCP binding site affinities and rotarod activities in mice and minimal motor impairment in rats (Vignon et al. 1982; Wallach 2014). The LD_{50} of PCP was determined to be 283 μmol/kg in mice (ip) (Vaupel et al. 1984). PCP has been shown to produce pro-convulsant effects at doses ($ED_{50} > 12$ mg/kg) greater than those which block electrically induced convulsions in female Sprague-Dawley rats (Leccese et al. 1986).

PCP has been observed to be self-administered in animal models including rats, beagle dogs, and rhesus monkeys (Aniline and Pitts 1982; Risner 1982; Marquis and Moreton 1987). Interestingly, PCP generalized to ethanol in drug discrimination studies in rats (Hundt et al. 1998; Balster and Chait 1976). Human volunteers with a history of alcohol dependence also describe alcohol-like effects following ketamine administration (Krystal et al. 1998). Furthermore, ethanol acts as an NMDAR antagonist (Krystal et al. 2003). Tolerance to PCP and a withdrawal syndrome have been reported in various animal models (Balster and Chait 1976; Balster and Woolverton 1980). Reports of tolerance in humans also exist although it has not been formally studied (Stillman and Petersen 1979; Pradhan 1984).

It is unknown how metabolites might contribute to the pharmacological activity of PCP however several have shown activity in various animal models. The *para*-hydroxylated metabolite of PCP (piperidine ring) produced PCP-like stimulus in rats trained to differentiate PCP from saline although the potency dropped to about 37%. 4-Hydroxylation at the cyclohexane ring reduced potency to 3% (Shannon 1981b). Both metabolites may possess reinforcing properties based on intravenous self-administration studies in beagle dogs although the potency relative to PCP was low (3% and 6%) (Risner 1982).

2.4 Clinical Toxicology

A large body of literature exists describing intoxications and fatalities involving PCP and a particularly relevant contribution to harmful effects and death include behavioral abnormalities that might lead to significantly impaired judgment and high-risk behavior (Cravey et al. 1979; Aniline and Pitts 1982; Showalter and Thornton 1977).

In a prospective study of 1,000 episodes of acute PCP intoxication (predominant route of administration was smoking), and with 60% of cases being attributed to PCP alone, a variety of clinical features were recorded, including nystagmus and hypertension. Severe alterations in vital signs were uncommon in patients who smoked only PCP. Forty-six percent of patients were alert and oriented, but cases of coma and excitement were also observed. Violent behavior (35%), agitation (34%), and bizarre behavior were encountered in 29% of cases (McCarron et al. 1981). Common findings from intoxication with PCP include a "drunken state," disorientation, catatonic effects, delusions, and hallucinations (Aniline and Pitts 1982; Showalter and Thornton 1977). High doses have been reported to lead to respiratory depression, coma, seizure activity, and fatality. Prolonged psychosis has been reported in some chronic users (Aniline and Pitts 1982; Bey and Patel 2007). The concentrations detected in various biofluids have been reported to vary substantially (Aniline and Pitts 1982; Walberg et al. 1983; Barceloux 2012), which makes the ability to identify direct correlations between concentration and drug effect challenging.

3 4-MeO-PCP

The publication of 4-MeO-PCP (Fig. 2a) synthesis dates back to at least the early 1960s as part of a systematic search for PCP analogs with potential clinical use (e.g., Anonymous 1960; Maddox et al. 1965). The first signs of underground 4-MeO-PCP experimentation began to surface in 1999, but it was not until about 2008 that 4-MeO-PCP arrived in online shops as one of the first research chemical dissociative drugs (Morris and Wallach 2014). The first notification of its detection within the European early warning system (EU-EWS) was reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in early 2011 (EMCDDA–Europol 2012).

4-MeO-PCP is active via the oral and parenteral route and is reported to induce dissociative effects but with substantially reduced potency relative to PCP and the positional isomer 3-MeO-PCP. Users generally describe active doses in the 50–100 mg range, which appears to be at least an order of magnitude below that of PCP and 3-MeO-PCP (Anonymous 2008). Analytical characterizations of various PCP and PCPy analogs and positional isomers including 4-MeO-PCP have been described (e.g., Costa and Speaker 1983; Brine et al. 1984; Ohta et al. 1987; Gole et al. 1988c; Casale 2011; Wallach et al. 2014).

3.1 Pharmacokinetics

Detailed pharmacokinetic investigations involving 4-MeO-PCP could not be identified at the time of writing, but the case report literature suggests that 4-MeO-PCP, similar to closely related analogs, can be identified in several biological matrices in the form of the parent species without having to rely solely on the detection of metabolites. Since 3-MeO-PCP biotransformation was revealed

to result, among others, in the formation of the *O*-demethylated product (3-HO-PCP) (Michely et al. 2017), further studies are warranted to confirm whether this metabolic conversion is also observed to any significant extent with 4-MeO-PCP. Although previously considered unclear (e.g., Gole et al. 1988a, b; Holsztynska and Domino 1985), the formation of the phenolic 4-HO-PCP metabolite derived from PCP has been identified in an in vitro study using human and rat liver microsomes and recombinant P450 2B incubations (Driscoll et al. 2007), and the extent to which this metabolite might occur with both PCP and 4-MeO-PCP in vitro deserves further study. Notably, 4-HO-PCP was reported to be a reactive material which underwent rapid decomposition under experimental conditions to yield a reactive quinone methide intermediate capable of reacting with nucleophiles including water and glutathione (GSH) (Driscoll et al. 2007).

3.2 Pharmacodynamic Effects In Vitro

4-MeO-PCP has shown modest binding affinity (Table 1) at the PCP binding site of NMDAR (Wallach 2014; Roth et al. 2013; Vignon et al. 1982; Kamenka and Geneste 1983). Its NMDAR affinity is lower than that obtained for PCP but more comparable (although still reduced slightly) to that determined for ketamine (Table 1). This reduced NMDAR affinity is also consistent with reports of its lower potency in humans compared to PCP and 3-MeO-PCP (Morris and Wallach 2014). Other recent investigations using human recombinant GluN1/N2A and GluN1/N2B preparations and the FLIPR/Ca^{2+} assay confirmed that 4-MeO-PCP was able to demonstrate antagonist properties at both NMDAR subtypes ($pIC_{50} = 4.8$ and 5.4) at reduced potency compared to PCP ($pIC_{50} = 5.9$ and 6.4) and ketamine ($pIC_{50} = 5.3$ and 5.9, respectively) (Zarantonello et al. 2011).

The structure activity relationship of arylcyclohexylamines has been fairly well characterized. Changes in the aromatic region can have dramatic impact on NMDAR affinity. For example, electron-donating substituents in the 2-, and 4-position generally lower NMDAR affinity, whereas electron-donating groups located at the 3-position of the phenyl ring either increase or do not reduce affinity relative to PCP. Electron-withdrawing substituents (e.g., fluorine) reduce potency relative to PCP in all positions although are generally better tolerated in the 3-position (Wallach 2014). However, a variety of 3,4-disubstituted analogs were found to retain affinity compared to PCP ($K_i = 57.9$ nM), such as 3,4-MD-PCP ($K_i = 62.8$ nM) or 3-Me-4-F-PCP ($K_i = 44.2$ nM). On the other hand, 3-Me-4-MeO-PCP displayed a reduction in affinity ($K_i = 185.3$ nM) (Fig. 2b), whereas it was still around three times higher compared to 4-MeO-PCP (Wallach 2014) (Table 1). Replacement of the phenyl ring for thiophene as in TCP and tiletamine increases NMDAR affinity and in vivo potency whereas many other aryl substitutions such as naphthyl, benzyl-, or aliphatic replacements led to loss of potency (Wallach 2014). Notably, Kalir reported 4-MeO-PCP (40% PCP) to be more potent than 2-MeO-PCP (30% PCP), though specific experimental details about how "central potency" was determined were not reported (Kalir 1981). 2-MeO-PCP did not induce dissociative effects at a dose of

12 mg (oral as HCl salt). A subsequent 18 mg dose induced only subtle effects although which appeared dissociative in nature (personal communication).

In addition to NMDAR affinity, 4-MeO-PCP has shown affinity at the human monoamine transporters SERT and NET but not DAT expressed in HEK293 cells ($IC_{50} > 10{,}000$ nM) (Table 1) and the sigma-1 ($K_i = 296$ nM and 517 nM) and sigma-2 receptors ($K_i = 143$ nM and 326 nM) (Roth et al. 2013; Wallach 2014). PCP ($IC_{50} = 500$ nM) and a range of other PCP analogs including 4-MeO-PCP ($IC_{50} = 3890$ nM) were shown to inhibit [^{3}H]DA uptake into rat brain synaptosomes (Vignon et al. 1988). 4-MeO-PCP also had weak affinity toward the alpha-2C adrenergic receptor subtype ($K_i = 5{,}214$ nM) (Wallach 2014).

Similar to PCP, 4-MeO-PCP displayed competitive butyrylcholinesterase and acetylcholinesterase inhibition, and potentiation of smooth muscle contractions in guinea-pig ileum preparations (Maayani et al. 1974). Binding experiments carried out with membrane preparations high in acetylcholine receptor density obtained from the electric organ of *Torpedo ocellata* demonstrated that a number of analogs modified at the phenyl ring (including 4-MeO-PCP, $K_i = 1{,}930$ nM) had comparable affinities (Haring et al. 1983). Electrophysiological studies to probe the impact of a number of PCP analogs including 4-MeO-PCP on nerve-evoked end-plate current (EPCs) recorded from the frog sartorius muscle revealed time- and voltage-dependent decreases in EPC amplitude (Aguayo and Albuquerque 1986). In an ex vivo model of protection, 4-MeO-PCP blocked *N*-methyl-D-aspartate (NMDA) (10 μM), and kainic acid (KA) (20 μM) mediated cell death in rat pup hippocampal slices with IC_{50} values of 4.65 and 42.95 μM, respectively (Wallach 2014). This protection against KA is intriguing as a comparably potent action was not seen with most other arylcyclohexylamines evaluated in this system including 3-MeO-PCP (Wallach 2014).

3.3 Effects In Vivo

Maddox and co-workers reported that 4-MeO-PCP was among the most active derivatives with cataleptic activity following intramuscular injections in pigeons. Although details were not reported, maximum activity for PCP, 4-MeO-PCP, and other derivatives were found at the 6–25 mg/kg level. On the other hand, 4-MeO-PCP was not among the most active drugs for antagonizing the tonic hindlimb extension in the MES test at least not in the range of 3–12.5 mg/kg (Maddox et al. 1965). Data published by Kalir et al. (1969) suggested that 4-MeO-PCP (at 10 mg/kg) did neither impact rotarod performance nor a conditioned avoidance response in albino mice and albino rats respectively. Digging behavior displayed by gerbils with 4-MeO-PCP (5 mg/kg) was not affected, and behavioral changes in cats (200 μg intraventricular injection) were also not observed. TCP, however, emerged as the most active compound during these studies (Kalir et al. 1969). Data published later, however, indicated that 4-MeO-PCP did impair rotarod performance in mice at a potency of about 25% relative to PCP (ED_{50} 11.8 mg/kg vs. 3.00 mg/kg, respectively) (Kalir et al. 1978). In contrast to PCP, 4-MeO-PCP did not induce mydriasis in mice and guinea pigs (Maayani et al. 1974). 4-MeO-PCP

was confirmed to cause impairment in the rotarod assay (subcutaneous administration) where the ED_{50} was reported to be 20 mg/kg in mice (male and female albino ICR), which was 5- and 4.17-fold less potent than PCP and 3-MeO-PCP, respectively (Vignon et al. 1982; Kamenka and Geneste 1983). 4-MeO-PCP was found to have activity in vivo at 30 mg/kg (but not 1, 3, or 10 mg/kg) in the MES test, consistent with its potency as an NMDAR antagonist (Wallach 2014). 4-MeO-PCP induced rotarod impairment in male CF-1 mice (intraperitoneal, ip) at 30, 100, and 300 mg/kg (Wallach 2014). Potency of 4-MeO-PCP in the MES and rotarod tests was reduced compared to 3-MeO-PCP and that generally reported for PCP (Wallach 2014). Notably, deaths were observed in male CF-1 mice at 100 and 300 mg/kg doses (ip), and 4-MeO-PCP was inactive (ip) against subcutaneous metrazole-induced seizure at doses from 1 to 30 mg/kg in mice. In contrast 3-MeO-PCP did show activity in this experimental model (Wallach 2014). The related compound and potential metabolite, 4-HO-PCP showed a significant loss in activity compared to PCP ([^{3}H]PCP $K_i = 20{,}000$ nM, $ED_{50} = 28$ mg/kg in rotarod test in mice), which represented a ~667- and ~13-fold reduction compared to 3-HO-PCP for affinity and rotarod potency, respectively (Vignon et al. 1982).

3.4 Clinical Toxicology

What appears to be the first published report of suspected 4-MeO-PCP intoxication involved a 45-year-old male with psychiatric history and emerged in 2012 with clinical features including disorientation, hypersalivation, tremors and occasional myoclonic jerks, scanning speech with dysarthria, and nystagmus in all directions of lateral gaze. The patient was reported to slowly respond to commands. Notably however the patient also ingested ethanol. The analytical confirmation of 4-MeO-PCP could not be obtained and was based on the self-report of the user (Misselbrook and Hamilton 2012).

A fatality associated with 4-MeO-PCP and the serotonergic hallucinogen 4-hydroxy-*N*-methyl-*N*-ethyltryptamine (4-HO-MET) was presented in 2015 where a 54-year-old man with a history of mental health issues, drug abuse, and hypertension was found dead as a consequence of mixed drug intoxication. The peripheral blood concentration of 4-MeO-PCP was determined at 8,200 ng/mL (central blood concentration was 14,000 ng/mL). Liver and urine concentrations were determined to be 120 mg/kg and 140 mg/L. An amount of 280 mg 4-MeO-PCP was also detected in the gastric contents suggesting oral ingestion of a high dose. Prescription drugs including venlafaxine, olanzapine, lorazepam, and hydroxyzine were also detected at therapeutic concentrations (McIntyre et al. 2015).

A number of intoxications involving 3-MeO-PCP and 4-MeO-PCP were reported from Sweden as part of the STRIDA project beginning in early-mid-2013. Intoxications were reported to resemble those of other dissociative drugs, but co-exposure to other NPS and/or classical "drugs of abuse" was frequently observed. Serum concentrations of 4-MeO-PCP in these cases were generally below 200 ng/mL, although in one case, a higher concentration (705 ng/mL) was detected (17–705 ng/mL, mean: 178, median: 131 ng/mL). Urine concentrations of 4-MeO-

PCP ranged from 61 to 71,673 ng/mL (mean: 14,979, median: 6506 ng/mL). Clinical features included hypertension, tachycardia, altered mental status (confusion, disorientation, dissociation, and/or hallucinations), agitation, and nystagmus among others. Cases involving only 4-MeO-PCP, however, were not described independently and most cases involved 3-MeO-PCP (Backberg et al. 2015).

The analysis of postmortem peripheral blood and urine specimens was reported from a case involving an accidental multiple drug-induced fatality in a 31-year-old male. The results revealed the detection of the synthetic opioids tetrahydrofuranylfentanyl (blood, 339 ng/mL; urine, >5,000 ng/mL) and U-49900 (blood, 1.5 ng/mL; urine, 2.2 ng/mL) but also an unspecified isomer of "MeO-PCP" (blood, 1.0 ng/mL; urine, 31.8 ng/mL). Alprazolam (10 ng/mL), paroxetine (10 ng/mL), topiramate (6,500 ng/mL), zolpidem (8.6 ng/mL), trazodone (360 ng/mL), aripiprazole (170 ng/mL), chlorpheniramine (63 ng/mL), dextro/levomethorphan (46 ng/mL), and promethazine (27 ng/mL) were also detected in blood, reflecting the individual's history of being treated for psychiatric disorders (Krotulski et al. 2017).

4 3-MeO-PCP

The synthesis and/or analytical characterizations of 3-MeO-PCP have been described on several occasions (e.g. Geneste et al. 1979; Brine et al. 1984; Ohta et al. 1987; Carroll et al. 1988; Haradahira et al. 1998; Zarantonello et al. 2011; De Paoli et al. 2013; Wallach et al. 2014), and the preparation of the ^{11}C-labeled counterpart via ^{11}C-methylation of 3-HO-PCP was published by Haradahira et al. (1998). Initial descriptions on the psychoactive effects of 3-MeO-PCP featured on the Internet appeared slightly later following those of 4-MeO-PCP. This was then followed by enthusiastic endorsement of the former compound by research chemical *aficionados*, which then culminated in a concerted research effort coordinated and executed by members of online communities to design, characterize, and explore a variety of arylcyclohexylamines including 3-MeO-PCP, 3-MeO-PCE, 3-MeO-PCPr, and 2-oxo-PCE, but also several new compounds such as 3-MeO-PCPy and MXE. Consistent with scientific research studies on a range of phenyl ring substituted PCP derivatives, the presence of the electron-donating methoxy group in the *meta*-position (3-position) increased potency compared to the *para*-position (4-position). 3-MeO-PCP is active via oral and parenteral routes and induces dissociative effects starting at around 5 mg although much higher doses are often reported (Morris and Wallach 2014). It appears that at present 3-MeO-PCP might be among the more popular representatives of the simple arylcyclohexylamine NPS based on availability, online discussion board postings, and the extent of reported intoxications in the literature. Notification of the first detection of 3-MeO-PCP by members of the EU-EWS was received by the EMCDDA in 2012 (EMCDDA–Europol 2013).

4.1 Pharmacokinetics

A systematic study regarding the biotransformation of 3-MeO-PCP was presented by Michely et al. (2017), who employed rat and pooled human liver microsomes but also included the identification of the cytochrome P450 (CYP) isoenzymes involved using human recombinant isozymes. Furthermore, the metabolic fate in male Wistar rats using urinalysis was investigated. 3-MeO-PCP was found to undergo extensive metabolism including multiple aliphatic hydroxylations at the cyclohexyl ring and piperidine ring, single aromatic hydroxylation, carboxylation after ring opening, *O*-demethylation, and glucuronidation. Hydroxylation reactions at the cyclohexyl ring were catalyzed by CYP2B6, whereas piperidine hydroxylations were catalyzed by CYP2B6 and CYP2C19. *O*-Demethylation was catalyzed by CYP2C19, CYP2B6, and CYP2D6 (Michely et al. 2017). One of the metabolites of particular interest is 3-HO-PCP, which is also a research chemical available for sale at this time (Sect. 6 below). A tentative identification of the *O*-demethyl species 3-HO-PCP together with confirmed identification of 3-MeO-PCP in postmortem femoral blood has been presented by Bakota et al. (2016) although it is unknown whether this could have also arisen from 3-HO-PCP consumption as well. The detection of three 3-MeO-PCP metabolites in human urine samples tentatively suggesting hydroxylations at the piperidine and cyclohexyl rings have also been reported (Zidkova et al. 2017). Although controlled clinical studies have not investigated the pharmacokinetics of 3-MeO-PCP in humans, an elimination $T_{1/2}$ was estimated to be ~11 h based on the analysis of four blood samples taken during the course of a hospitalization case (Johansson et al. 2017). Similarly, Backberg et al. (2015) estimated an elimination $T_{1/2}$ of ~10 h based on the analysis of two blood samples taken from an intoxication case.

4.2 Pharmacodynamic Effects In Vitro

Available data suggest that 3-MeO-PCP shows slightly higher affinity toward the PCP site of NMDAR than PCP. Correspondingly, NMDAR affinity was reported to be an order of magnitude higher than 4-MeO-PCP (Table 1). 3-MeO-PCP has also been reported to have affinities for DAT, SERT, and NET (Table 1), but some discrepancies exist between the studies (Roth et al. 2013; Wallach 2014; Vignon et al. 1988). The affinity values determined for the sigma-1 receptor under identical screening conditions were $K_i = 42$ nM (Roth et al. 2013) and $K_i = 436$ nM (Wallach 2014), respectively, and whereas an $IC_{50} > 10{,}000$ nM was reported by Roth et al. (2013) for sigma-2 receptor affinity, a K_i value of 154.4 nM was reported by Wallach (2014). In the study reported by Vignon et al. (1982) and Kamenka and Geneste (1983) ([^{3}H]PCP as radioligand), the affinity of 3-MeO-PCP increased ~2.8-fold relative to PCP (Table 1), whereas 2-MeO-PCP and 4-MeO-PCP had reduced affinity by a factor of 2 and 4.8, respectively. A recent investigation obtained a K_i value of 147.5 nM for 2-MeO-PCP ([^{3}H]MK-801 in rat forebrain) (Wallach and Colestock unpublished), which was 3.87-times lower than 3-MeO-PCP but 4.2-

times higher than 4-MeO-PCP under identical conditions (Wallach 2014). 3-MeO-PCP (IC_{50} = 490 nM) and PCP (IC_{50} = 500 nM) had comparable potency on inhibition of [^{3}H]DA uptake into rat brain synaptosomes although 3-MeO-PCP was ~eightfold more potent than 4-MeO-PCP (Table 1) (Vignon et al. 1988). In an ex vivo model of neuroprotection, 3-MeO-PCP blocked NMDA (10 μM), and kainic acid (20 μM) mediated cell death in rat pup hippocampal slices with IC_{50} values of 0.381 and 208.1 μM, respectively. In comparison, the IC_{50} values measured for 4-MeO-PCP were 4.65 and 42.95 μM, respectively (Wallach 2014). In one study, 3-MeO-PCP had fairly high affinity at the kappa opioid receptor (KOR, K_i = 168.8 nM) (Wallach 2014) but lower affinity at MOR (K_i = 9,418 nM) (Wallach 2014). However this was not seen in Roth et al. (2013) using equivalent assays.

The related compounds 3,4-MD-PCP (NMDAR K_i = 62.8 nM) and 3-Me-PCP (NMDAR K_i = 34.8 nM) (Fig. 2) also have high affinities for NMDAR with modest to low affinities at monoamine transporters (Table 1), and all show affinities at sigma-1 and sigma-2 (Wallach 2014). These compounds illustrate that electron-donating substituents in the 3-position led to an increase or did not alter affinity for NMDAR relative to PCP. 3,4-MD-PCP is active in humans inducing dissociative effects via parenteral and oral routes at doses from 5–20 mg (HCl salt). Likewise, 3-Me-PCP is an active dissociative (parenteral and oral routes) at 5–10 mg (HCl salt) (personal communication). The high potency of these compounds is consistent with their high affinities for the PCP binding site of NMDAR.

4.3 Effects In Vivo

3-MeO-PCP was found to potently inhibit the tonic hindlimb extension in the MES test (male CF-1 mice MES ip ED_{50} = 3.2 mg/kg and oral (po) ED_{50} = 6.6 mg/kg and male Sprague-Dawley rat ip ED_{50} = 4.05 mg/kg and po ED_{50} = 3.2 mg/kg). Affinities for the PCP binding site of NMDAR and NMDAR antagonism potency correlated well with potency in the MES test for a series of arylcycloalkylamines (Wallach 2014; Wallach unpublished). Likewise, 3-MeO-PCP caused rotarod impairment in male CF-1 mice (ip, ED_{50} = 5.2 mg/kg and po, ED_{50} = 11.29 mg/kg) and minimal motor impairment in male Sprague-Dawley rats (ip, ED_{50} = 5.94 mg/kg, po, ED_{50} = 15.3 mg/kg). This motor impairment was typically seen at doses slightly higher compared to those found to exert anticonvulsant effects (Wallach 2014). Notably, the protective index (MES ED_{50}/rotarod ED_{50}) of 3-MeO-PCP was slightly higher in these models than that reported for PCP but not as high as some other derivatives (e.g., 3-MeO-PCPy) (Wallach 2014). Similar to observations made with the MES test, rotarod potencies showed high correlation with PCP binding site affinities (Wallach 2014). Comparable rotarod potency in mice was reported in another study with an ED_{50} of 4.8 mg/kg, which was only slightly less potent than PCP (4 mg/kg) and 4.17-fold more potent than 4-MeO-PCP (Vignon et al. 1982; Chaudieu et al. 1989). 3-MeO-PCP also showed protection against metrazole-induced seizures in male CF-1 mice (partial protection at 20 mg/kg, po) and rats (po, ED_{50} = 9.4 mg/kg). During evaluation of anticonvulsant and rotarod testing at

the 100 mg/kg (ip), mice were described as sedated, and some test subjects died at 100 and 300 mg/kg doses (Wallach 2014).

4.4 Clinical Toxicology

Three deaths associated with 3-MeO-PCP that occurred in Sweden were reported to the EMCDDA in October 2014 (EMCDDA–Europol 2015). An attempted murder case was reported in which a male drug user with a history of drug use and drug-induced psychosis was reported to have snorted "large quantities" of 3-MeO-PCP and MDPV (a psychostimulant NPS), in addition to inhaling butane gas. Clinical features of the intoxication included vivid visual, auditory, and tactile hallucinations and bizarre ideas. Notably, the hallucinations were reported to take 6 weeks to fully resolve. It was unclear whether this might have been related to the history of drug-induced psychosis, which had been treated with risperidone, which he stopped taking prior to this event due to side effects. Analytical confirmation from the analysis of biofluids, however, was not presented (Stevenson and Tuddenham 2014).

In an evaluation of data collected as part of the Swedish STRIDA project, 56 patients tested positive for 3-MeO-PCP and 11 for 4-MeO-PCP in the period between July 2013 and March 2015, and eight of these cases involved both substances (Backberg et al. 2015). The detection of 3-MeO-PCP alone (not involving other drugs) was encountered in only seven cases, and the clinical features noted in these instances included hypertension, tachycardia, confusion, disorientation, dissociation, and hallucinations. Two intoxications were considered severe (poisoning severity score (PSS) = 3), four as moderate (PSS = 2), and one as mild (PSS = 1). The concentrations of 3-MeO-PCP in serum were found to be generally below 110 ng/mL, albeit one case revealed a concentration of 242 ng/mL (Backberg et al. 2015).

A postmortem analysis of blood samples obtained from a fatal intoxication case of a 29-year-old male revealed a 3-MeO-PCP concentration of 139 ng/mL together with 4.1 mg/L diphenhydramine, a marijuana metabolite (presumptively positive), and amphetamine below 100 ng/mL. Congested lungs and distended bladder indicating urinary retention and moderate atherosclerosis were also noted during autopsy (Bakota et al. 2016).

Two nonfatal intoxications involving young males (19 and 21 years), exhibiting a comatose state (Glasgow coma score = 3), respiratory acidosis, right anisocoria, mydriatic pupils and hypothermia, required hospitalization. A delirious and agitated state, including euphoria was still noted in one case 24 h after hospitalization. Blood-alcohol concentrations were 2.0 and 1.7 g/L. Measured blood and urine concentrations were 350.0 and 6,109.2 ng/mL and 180.1 and 3,003.6 ng/mL, respectively (Bertol et al. 2017).

Fifteen minutes after consuming 3-MeO-PCP (routes of administration not reported), two males (37 and 40 years) were reported to have experienced "disorientation, hallucinations, spastic leg postures, and facial grimacing." In the first case (~2 h after drug ingestion), hypertension, tachycardia, and signs of psychotic behavior and altered mental status (Glasgow coma scale = 10–12) but

also increased muscle tone with spastic leg postures were noted. Serum concentration of 3-MeO-PCP, 2 h after drug ingestion, was 49 ng/mL. In addition to methamphetamine (121 ng/mL) and amphetamine at 10 ng/mL were detected. The patient was discharged 24 h after hospital admission and reported complete amnesia during the period of intoxication. In the second case, the patient was described with a deteriorating state of consciousness (Glasgow coma scale = 12), hypertension, and tachycardia. The medical history included diabetes mellitus with chronic renal failure and a renal transplant 2 years previously. Current medication included prednisone, tacrolimus, and mycophenolate mofetil. Serum concentration of 3-MeO-PCP measured, approximately 2 h after drug ingestion, was 66 ng/mL. Complete amnesia was reported during the time of intoxication, and the patient was discharged 8 h after hospital admission. Both subjects were administered naloxone and flumazenil during transport to the hospital with no noticed effect. Three hydroxylated metabolites of 3-MeO-PCP tentatively identified as piperidine and cyclohexane ring transformation products were identified in urine of both patients (Zidkova et al. 2017).

A nonfatal intoxication and seven deaths (March 2014–June 2016) involving 3-MeO-PCP were reported in Sweden (Johansson et al. 2017). The nonfatal intoxication (19-year-old male) involved a drug user found in a catatonic state at home (Glasgow coma scale = 11) whose clinical features were described as tachycardia, hypertension, and tachypnea. The patient also developed pyrexia and lactic acidosis, became agitated and began to hallucinate, which triggered treatment with diazepam and haloperidol followed by propofol and intubation after the patient began to snore and exhibited reduced oxygen saturation. The concentrations of 3-MeO-PCP in whole blood obtained from various sampling intervals were 0.14 μg/g at admission to the emergency department, 0.08 μg/g 2.5 h after admission, 0.06 μg/g 5 h after admission, and 0.04 μg/g 17 h after admission. The fatal cases involved six males and one female (age range 20–32, mean 26, median 27), all found dead at home. The causes of death were deemed accidental in five cases and suicide in two of the cases. The femoral blood concentrations of 3-MeO-PCP ranged between 0.05 and 0.38 μg/g (postmortem interval 48–244 h). Other drugs were identified in six out of seven cases (Johansson et al. 2017).

A 26-year-old male with a history of substance use disorder presented with generalized hypertonia, ocular revulsion, and contact rupture following the ingestion of multiple drugs on the same day. In addition to his daily therapy which included, oxazepam 50–150 mg/day, venlafaxine 37.5 mg and 80 mg methadone orally (regular therapy) he claimed to have taken 1 g MDMA by several injections of 100 mg and 20–30 "reefers" (joints) of AB-FUBINACA (synthetic cannabinoid receptor agonist) and 200 mg 3-MeO-PCP. Intensive care treatment involved midazolam and propofol administration. The patient fell into coma and required mechanical ventilation for 4 days. Analysis of blood and urine samples and drug paraphernalia confirmed the detection of AB-FUBINACA, 3-MeO-PCP, methadone, MDMA, venlafaxine, and benzodiazepine (details not reported) (Lomenech et al. 2017). In a related publication, it was stated that the same individual believed that he had consumed 4-MeO-PCP, which, following analysis of powdered sample

and biofluids, transpired to be the 3-MeO-PCP isomer (concentration not reported). Quantitative blood results for the following substances were identified: methadone = 278 ng/mL, venlafaxine = 190 ng/mL, oxazepam = 33 ng/mL, and MDMA = 72 ng/mL (Allard et al. 2017).

A case involving an intoxication with the dissociative MXE and 3-MeO-PCP was described in a 27-year-old male with a history of attention deficit hyperactivity disorder, bipolar disorder, and hypertension. He reported taking prescribed lithium, methylphenidate, and trazadone. The patient was hypertensive and tachycardic and showed signs of dissociated affect, a delayed verbal response to questions, ataxia, and vertical nystagmus. Several blood samples were obtained, and detected concentrations were 279 ng/mL, 205 ng/mL, and 180 ng/mL for MXE and 167 ng/mL, 131 ng/mL, and 90 ng/mL for 3-MeO-PCP at 0, 2, and 3 h, respectively (Thornton et al. 2017).

Two deaths involving males (21 and 58 years) have been reported that revealed postmortem (peripheral/central) blood concentrations of 3,200 ng/mL and 630 ng/mL 3-MeO-PCP, respectively. In addition, methamphetamine (110 ng/mL) was detected in the 58-year-old. In addition, this patient was prescribed a number of medications that were not tested for. In the other case ethanol (0.047 g/100 mL), bupropion (1,800 ng/mL), delorazepam, paroxetine, and mitragynine (levels not determined) were also detected in blood (Mitchell-Mata et al. 2017).

The analysis of postmortem peripheral blood and urine specimens was reported that originated from an accidental multiple drug-induced fatality involving a 31-year-old male. The results revealed the detection of the synthetic opioids tetrahydrofuranylfentanyl (blood, 339 ng/mL; urine, >5,000 ng/mL) and U-49900 (blood, 1.5 ng/mL; urine, 2.2 ng/mL) but also an unspecified isomer of "MeO-PCP" (blood, 1.0 ng/mL; urine, 31.8 ng/mL). Alprazolam (10 ng/mL), paroxetine (10 ng/mL), topiramate (6,500 ng/mL), zolpidem (8.6 ng/mL), trazodone (360 ng/mL), aripiprazole (170 ng/mL), chlorpheniramine (63 ng/mL), dextro/levomethorphan (46 ng/mL), and promethazine (27 ng/mL) were also detected in blood, reflecting the individual's history of being treated for psychiatric disorders (Krotulski et al. 2017).

A 27-year-old male with a history of schizophrenia was hospitalized for 3-MeO-PCP intoxication (qualitatively confirmed with detection in urine sample). He had amnesia and reported feeling sedated and "loopy." He expressed delusions, for example, stating he "was an alien with green blood." Vital signs were normal except for borderline tachycardia, mild hypokalemia, hypophosphatemia, slightly increased aspartate aminotransferase, and indirect bilirubemia (Chang and Smith 2017).

5 3-MeO-PCPy

In contrast to PCPy, which had a history in scientific research and illicit street drug presence, 3-MeO-PCPy does not appear to have been described in the scientific literature prior to its inception and development by members of an online discussion

forum (Morris and Wallach 2014), and as such the available information is more limited. The synthesis and analytical characterizations has been described (Wallach et al. 2014). 3-MeO-PCPy does not appear to have ever reached the popularity of 3-MeO-PCP or other dissociative NPS such as MXE although it is an active dissociative in humans at 5–10 mg (HCl salt) and is active after parenteral and oral routes of administration and was described as "stimulating" (personal communication).

5.1 Pharmacokinetics

Together with 3-MeO-PCP, an extensive metabolism study was undertaken with 3-MeO-PCPy that included incubations with rat and pooled human liver microsomes, administration in male Wistar rats for urinalysis, and exposure to human recombinant cytochrome P450 isoforms (Michely et al. 2017). As with 3-MeO-PCP, 3-MeO-PCPy also underwent extensive biotransformation including multiple aliphatic hydroxylations at the heterocyclic and cyclohexyl rings, carboxylation after ring opening, single aromatic hydroxylation, *O*-demethylation, and glucuronidation. In contrast to 3-MeO-PCP, the metabolite resulting from a single hydroxylation at the cyclohexyl ring was not detected. Lactam formation was another feature observed for 3-MeO-PCPy that was not observed with 3-MeO-PCP. Experiments with recombinant isozymes revealed that *O*-demethylation was catalyzed by CYP2D6, CYP2C9, and CYP2B6, whereas pyrrolidine ring hydroxylation was catalyzed by CYP2B6 (Michely et al. 2017).

5.2 Pharmacodynamic Effects In Vitro

3-MeO-PCPy was found to have high affinity at the PCP site of NMDAR in rat forebrain (K_i = 22.3 nM), as well as at human monoamine reuptake transporters NET (K_i = 96 nM), and SERT (K_i = 11 nM) expressed in HEK293 cells. However, 3-MeO-PCPy lacked significant affinity (IC_{50} > 10,000 nM) at DAT under the experimental conditions used (Table 1). In addition, receptor screening results also revealed a K_i value of 2,398 nM at the alpha-2A adrenergic receptor subtype. High affinity for monoamine sites appears common with a number of pyrrolidine containing arylcyclohexylamines (Wallach 2014). For example, the related compounds 3-Me-PCPy and 3,4-MD-PCPy (Fig. 2b) have also been synthesized and investigated, although so far they have not appeared as research chemicals offered for sale. Both showed high affinity for the PCP binding site of NMDAR (Table 1) consistent with electron-donating substituents located at the *meta*-position (K_i = 52.3 and 46.9 nM for 3-Me- and 3,4-MD-PCPy, respectively). Both compounds are reported to be active in humans with dissociative effects starting at doses around 5 mg (as HCl salts) through nasal insufflation with a several hour duration (personal communication).

Notably, 3-Me-PCPy showed high affinities for DAT, NET, and SERT comparable to or higher than its NMDAR affinity (39, 45, 5.6, and 52.3 nM, respectively), which was not shared by its PCP counterpart 3-Me-PCP. Furthermore, 3-Me-PCPy was also shown to act as a reuptake inhibitor at these transporters (Wallach 2014). 3,4-MD-PCPy lacked significant affinity at DAT ($IC_{50} > 10{,}000$ nM) but showed modest affinity at NET and SERT ($K_i = 420$ and 115 nM). This is a similar trend to that seen with 3-MeO-PCPy. The substituted PCPy series generally were found to have higher affinities at monoamine reuptake transporters than their PCP counterparts (Wallach 2014). Interestingly, some users have reported 3-Me-PCPy to show a notable psychostimulant activity in addition to dissociative effects, which suggests that this might be an unusual feature compared to structurally related compounds. Interestingly, PCPy has been said to have sedative effects similar to barbiturates (Shulgin and MacLean 1976). Four and eight mg doses (nasal insufflation of HCl salt) were reported to induce a relaxing ethanol-like state where sleep was possible (a state uncommon with most other arylcyclohexylamines which tend to be slightly stimulating especially at lower to medium doses) although it was reported to clearly represent a dissociative effect and distinct in nature from the effects induced by classic GABAergics including benzodiazepines and barbiturates (personal communication). Efforts should be made to further investigate the potential pharmacological reasons for these apparently unique effects. For comparison purposes, the IC_{50} values obtained from NMDAR binding studies of PCPy were 290 nM ([^{3}H]-MK-801, rat cortex homogenate) (Stefek et al. 1990), 140 nM ([^{3}H]PCP, rat brain homogenate) (Kozlowski et al. 1986), and 200 nM ([^{3}H]PCP, rat brain homogenate) (Zukin and Zukin 1979). Displacement studies of [^{3}H]PCP-specific binding in rat olfactory bulb slices revealed PCPy ($IC_{50} = 65$ nM) to be more potent than PCP ($IC_{50} = 90$ nM) and almost equipotent to TCP ($IC_{50} = 54$ nM). PCE was shown to be more potent ($IC_{50} = 15$ nM), whereas ketamine was much less potent ($IC_{50} = 800$ nM) (Quirion et al. 1981). When employing [^{3}H]PCP-specific binding to guinea-pig ileum preparations, the rank order of potency among a number of PCP analogs showed (IC_{50}): TCP (300 nM) > PCE (400 nM) > PCPy (450 nM) > PCP (500 nM) > PCMo (1,000 nM) > ketamine (5,500 nM) (Gintzler et al. 1982). However, it is unclear which receptor(s) were being labeled in this experiment, which makes interpretation of these particular results challenging.

5.3 Effects In Vivo

3-MeO-PCPy (po) prevented the tonic hindlimb extension in the MES test and caused rotarod impairment in male CF-1 mice ($ED_{50} = 8.03$ and 19.21 mg/kg, respectively). In male Sprague-Dawley rats (po), the MES ED_{50} and rotarod ED_{50} values were 11.04 and 17.39 mg/kg, respectively. Deaths were reported in mice at 100 and 300 mg/kg (ip). Furthermore, it was found that 3-MeO-PCPy showed protection against metrazole-induced seizures at doses from 10 to 30 mg/kg and protected (po) against 6 Hz seizure in male CF-1 mice (Wallach 2014).

The ring-unsubstituted analog PCPy was also active in the rotarod test (mice) and completely abolished (at 8 mg/kg) the conditioned avoidance response in rats, and

although less potent, this compared qualitatively with TCP. Both drugs disrupted digging ability in gerbils (Kalir et al. 1969). PCPy was also observed to produce PCP-like discriminative stimulus effects in rats (two-choice, shock escape-avoidance task) comparable to those produced by 3.0 mg/kg PCP (ip). In comparison, TCP was 1.31-fold more potent, whereas its pyrrolidine counterpart (TCPy) dropped to 87% of the PCP potency. PCMo and ketamine only displayed 10% potency relative to PCP whereas PCE was determined to be almost six times more potent than PCP in this assay (Shannon 1981b). In another study assessing PCP-like discriminative stimuli effects in rats, PCPy was found to show a 0.91-fold potency of PCP. Overall it was confirmed that substitutions on the nitrogen atom of PCP either increased or decreased potency but without affecting efficacy (Cone et al. 1984; Shannon 1981a). In a PCP discriminative stimulus test in pigeons, the potency of PCPy was slightly higher than that observed with PCP (McMillan et al. 1988).

PCPy was also found to be equipotent to PCP in producing increases in avoidance response rates in rats (electric shock avoidance), thus, confirming pharmacological similarities to PCP and other closely related analogs (Shannon and DeGregorio 1981). Studies in beagle dogs revealed that PCPy showed reinforcing properties with a potency of 27% of that of PCP (Risner 1982). In the squirrel monkey PCP, appropriate responding was produced in the following rank order (ED_{50}) for drug lever responding: TCP (0.036 mg/kg) > PCP (0.055 mg/kg) = PCE (0.058 mg/kg) > PCPy (0.07 mg/kg) > PCMo (0.37 mg/kg) > ketamine (0.48 mg/kg) (Brady and Balster 1981). In the rotarod test in mice, the PCPy (ip) ED_{50} value determined was 16.4 μmol/kg (PCP 17.6 μmol/kg), whereas the LD_{50} value was 206 μmol/kg (PCP LD_{50} = 283 μmol/kg) (Vaupel et al. 1984). PCPy, similar to PCP and other analogs, showed both pro- and anticonvulsive effects in female Sprague-Dawley rats (Leccese et al. 1986). In male Sprague-Dawley derived rats, PCPy induced behaviorial effects consistent with PCP including ataxia, headweaving, turning, backpedalling, sniffling and turning with more or less comparable potency to PCP in most of these responses (Cho et al. 1991). PCPy showed reduced potency relative to PCP in inducing a number of effects in dogs including tachycardia, mydriasis, analgesia, hypersecretion, hypernea, and flexor reflex depression, and PCPy was about half as potent as PCP in general PCP behavioral effects (Vaupel 1983).

5.4 Clinical Toxicology

Reports of acute toxicity associated with 3-MeO-PCPy could not be identified. Interestingly, an acute intoxication in a 31-year-old male induced by PCPy was reportedly successfully treated with three intramuscular injections of 2 mg physostigmine salicylate administered in 20 min intervals, which led to reversal of clinical features. In this case report, a 31-year-old male presented with horizontal nystagmus, resting tremor of the upper limbs, agitation, hostility, suspiciousness, panic, and thoughts of impending doom approximately 3–4 h following ingestion (Giannini and Castellani 1982). The application of 5 mg haloperidol (im) has also been reported to

resolve PCPy psychosis in 20 hospitalized male subjects when compared to placebo (Giannini et al. 1985).

6 3-HO-PCP

3-HO-PCP has been the subject of pharmacological research when it was discovered that it showed high affinity toward NMDAR and opioid receptor subtypes, with the potential contributions of the latter to the psychopharmacological profile warranting further studies. The syntheses and analytical characterizations have been described (e.g., Kalir et al. 1978; Kamenka et al. 1982; Brine et al. 1984; Gole et al. 1988c; Wallach 2014). 3-HO-PCP has been the topic of continuous discussion over the years in Internet discussion forums dedicated to the topic of arylcyclohexylamines, and it appears that its emergence as a research chemical was noticed around 2009. In addition to dissociative effects, some users report opioid-like effects with this compound, consistent with its pharmacology described below (Morris and Wallach 2014). 3-HO-PCP is reported to be active at doses from 1 to 10 mg in humans (Anonymous 2012a). A report involving analytically confirmed material found it fully active at 6 mg with nasal insufflation inducing classic dissociative effects with "opioid" activity (personal communication). Notably, unpleasant dissociative effects and muscle tension have been reported although not shared by others (Anonymous 2012b).

6.1 Pharmacokinetics

Detailed pharmacokinetic investigations employing 3-HO-PCP specifically could not be identified. However, as described above, 3-HO-PCP has been detected in rat urine and in incubations with rat and human liver microsomes as a metabolite of 3-MeO-PCP. Furthermore, CYP activity screening with human recombinant isozymes revealed that *O*-demethylation of 3-MeO-PCP was catalyzed by CYP2C19, CYP2B6, and CYP2D6 (Michely et al. 2017). Likewise, 3-HO-PCP was tentatively identified without confirmation with reference material in postmortem blood samples obtained from a fatal intoxication case associated with 3-MeO-PCP (Bakota et al. 2016). The extent to which 3-HO-PCP might arise from PCP metabolism in humans in significant quantities warrants further investigations (e.g., Gole et al. 1988a, b; Holsztynska and Domino 1985; Ohta et al. 1987).

6.2 Pharmacodynamic Effects In Vitro

3-HO-PCP was shown to be a potent NMDAR antagonist with typically higher affinity than that observed for PCP and 3-MeO-PCP (Table 1). In addition, 3-HO-PCP (IC_{50} = 1,360 nM) was reported to show an almost threefold reduced

ability to inhibit [^{3}H]DA uptake into rat brain synaptosomes when compared to PCP (IC_{50} = 500 nM) and 3-MeO-PCP (IC_{50} = 490 nM) (Chaudieu et al. 1989). In terms of binding to monoamine reuptake transporters, 3-HO-PCP lacked affinities for human NET and SERT (IC_{50} > 10,000 nM) and had only weak affinity for DAT (K_i = 1,154 nM) expressed in HEK293 cells. Affinities measured for additional targets included alpha-2A (K_i = 581 nM), alpha-2C (K_i = 3,311 nM), sigma-1 (K_i = 239 nM), and sigma-2 receptors (K_i = 395 nM) (Wallach 2014). Autoradiographic studies in rat brain tissue slices showed that the [^{3}H]3-HO-PCP binding site was identical to the PCP binding site also labeled by [^{3}H]TCP and [^{3}H]MK 801 (Suzuki et al. 1996). However, the high affinity seen at MOR suggests that this may not be the case.

In addition, 3-HO-PCP has been found to have potent affinity at MOR. For example, the IC_{50} values against [^{3}H]morphine binding in rat brain (minus cerebellum) was found to be 39 nM by comparison PCP, IC_{50} = 11,000 nM and morphine, IC_{50} = 2.8 nM. Functional activity experiments with 3-HO-PCP showed opioid-like inhibition in guinea pig ileum preparations which were naloxone-reversible. However, 3-HO-PCP also antagonized the inhibitory effects of morphine in guinea pig ileum, and morphine induced evoked contractions in mouse vas deferens suggesting mixed agonist-antagonist action, possibly suggesting a partial agonist profile (Itzhak et al. 1981a). The same group obtained a K_i value of 55 nM for 3-HO-PCP obtained from displacement of [^{3}H]morphine in rat brain (minus cerebellum) (For comparison PCP, IC_{50} = 13,000 nM; morphine, IC_{50} = 4.2 nM) (Itzhak et al. 1981b). Appreciable affinities have been determined for 3-HO-PCP using a number of radiolabeled ligands to label different opioid sites (and sigma receptors) in rat brain homogenates (minus cerebellum): K_i = 2,300 nM ([^{3}H]leucin enkephalin), K_i = 140 nM [^{3}H] ethylketocycloazocine, and K_i = 42 nM ([^{3}H]SKF-10047) (Itzhak et al. 1981a). Similar results have been reported by others (Kamenka et al. 1982; Johnson et al. 1984) and more recently by Wallach (2014) (MOR, K_i = 86 nM; KOR K_i = 1,096 nM).

6.3 Effects In Vivo

3-HO-PCP (ED_{50} = 1.24 mg/kg) was found to be more potent than PCP (ED_{50} = 3.00 mg/kg) and equipotent to PCE (ED_{50} = 1.25 mg/kg) in the rotarod test in male and female albino ICR mice (Kalir et al. 1978), which has been confirmed subsequently with ED_{50} values of 2.2 mg/kg for 3-HO-PCP and 4 mg/kg for PCP (sc) (Vignon et al. 1982; Kamenka et al. 1982; Kamenka and Geneste 1983). 3-HO-PCP caused motor impairment in the mouse platform test (ED_{50} = 9.40 μmol/kg, ip) with a relative potency to PCP of 1.1 (Domino et al. 1983). Regarding the interactions with potential MOR-related opioid activity, 3-HO-PCP was shown to have potent analgesic effects in vivo in a writhing test using acetic acid in male ICR mice with an ED_{50} of 1.3 mg/kg (sc). In comparison, morphine and PCP administration (sc) gave ED_{50} values of 0.42 and 2.8 mg/kg. The effect of 3-HO-PCP in the writhing test was reduced to 32% with naloxone (0.5 mg/

kg), again suggesting contributions via MOR to the analgesic effects (Itzhak et al. 1981b).

6.4 Clinical Toxicology

Reports of acute toxicity associated with 3-HO-PCP could not be identified. Should 3-HO-PCP be formed in humans, for example, from metabolic hydroxylation of PCP or metabolic demethylation of 3-MeO-PCP (Michely et al. 2017; Bakota et al. 2016), then pharmacological contributions from such a metabolite warrant further investigation.

7 3-MeO-PCE, 3-MeO-PCPr, and 3-HO-PCE

User discussions about the emergence of 3-HO-PCE started to appear in 2012 although the limited information available creates uncertainties about its psychopharmacological profile in users. Both 3-MeO-PCE and 3-MeO-PCPr arose from collaborative research efforts between individuals linked via an online discussion forum and appeared as research chemicals in 2010 and 2011, respectively (Morris and Wallach 2014). Both are fairly potent dissociatives in humans and are active via parenteral and oral routes with dissociative effects starting below 10 mg although higher doses are also commonly reported (Anonymous 2017, 2018b, personal communication). A notification about the detection of 3-MeO-PCE was received by the EMCDDA in November 2010 (EMCDDA–Europol 2011). The syntheses of 3-MeO-PCE, 3-MeO-PCPr, and 3-HO-PCE and some analytical and pharmacological characterizations including use in bioanalytical method development and investigations of positional isomers have been described (Reel et al. 1988; De Paoli et al. 2013; Dresen et al. 2014; Wallach et al. 2016b; Lehmann et al. 2017). A notification about the detection of 3-HO-PCE was received by the EMCDDA in November 2017 (EMCDDA, personal communication).

7.1 Pharmacokinetics

At the time of writing, information on pharmacokinetic parameters on 3-MeO-PCE, 3-MeO-PCPr, and 3-HO-PCE could not be identified. Further studies are warranted, in particular to confirm whether the analytical detection of 3-HO-PCE and 3-HO-PCPr would arise from biotransformations of the *O*-methylated parent drugs as is the case with some structurally related compounds.

7.2 Pharmacodynamic Effects In Vitro

Limited information is available about the pharmacology of 3-HO-PCE. One study published by researchers from Eli Lilly found high affinity for the PCP binding site (IC_{50} = 23 nM, [^{3}H]PCP, rat cortex). Data collected from the same experiments

revealed an IC_{50} value of 99 nM for 3-MeO-PCE, whereas 3-MeO-PCPr was not quantified but showed 53% displacement of [^{3}H]-PCP at a 100 nM concentration (93.6% at 1,000 nM). Similarly, 3-HO-PCPr, which might be revealed as a potentially active metabolite of 3-MeO-PCPr, was also found to have high PCP site binding affinity (IC_{50} = 39 nM) (Reel et al. 1988). More recently, 3-MeO-PCE was reported to have high affinity at the PCP binding site of NMDAR ([^{3}H]-MK-801, rat brain) in two studies, which uncovered K_i values of 61 nM (Roth et al. 2013) and 30.4 nM (Wallach 2014) (Table 1). 3-MeO-PCE also showed affinity for human SERT (K_i = 115 and 136 nM), DAT (K_i = 743 and 906 nM), and NET (IC_{50} > 10,000 nM and K_i = 1,528 nM) expressed in HEK293 cells. Also under identical screening conditions, the determined affinities for sigma receptor subtypes were K_i = 4,519 and 1,725 nM (sigma-1) and K_i = 525 and 636 nM (sigma-2), respectively (Roth et al. 2013; Wallach 2014; Roth et al. 2018). 3-MeO-PCPr was also confirmed to show an appreciable affinity (K_i = 17.9 nM) for the PCP site of NMDAR using [^{3}H]-MK-801 in rat forebrain (Table 1). In addition, 3-MeO-PCPr showed modest affinities for DAT (K_i = 381 nM), NET (K_i = 1,342 nM), SERT (K_i = 700 nM) (Table 1), sigma-1 (K_i = 1,579 nM), sigma-2 (K_i = 467.5), and KOR (K_i = 1,318 nM) (Wallach 2014).

Although apparently not available as research chemicals at the present, a number of other *N*-alkylated arylcyclohexylamines, such as 3-Me-PCMe, 3-Me-PCE, 3-MeO-PCMe, 3-Me-PCPr, 3,4-MD-PCE, and 3,4-MD-PCPr (Fig. 2b), have been investigated for their interaction with a number of biological targets. All have shown affinities toward the PCP binding site of NMDAR in rat forebrain and some monoamine reuptake transporters comparable to other analogs (Table 1). The only examples found to have some affinity for KOR were 3-MeO-PCPr (K_i = 1,318 nM), 3-Me-PCPr (K_i = 832 nM), and 3,4-MD-PCPr (K_i = 998 nM). These compounds did not show significant affinities for MOR (IC_{50} > 10,000 nM). Apart from 3,4-MD-PCPr that did not show > 50% displacement of radioligand at a concentration of 10,000 nM at sigma-1 receptor, all others showed affinities at the sigma-1 (K_i = 227–2,817 nM) and all showed sigma-2 binding site (K_i = 104–1,235 nM). Notably, 3,4-MD-PCPr was the most selective for the PCP binding site of NMDAR of a large series of arylcycloalkylamines evaluated (Wallach 2014) making it potentially valuable for further research requiring selective NMDAR antagonists and for investigations into structure activity relationship features that improve selectivity (Wallach 2014). Consistent with the observed NMDAR binding affinities some of these compounds have been found to be potent dissociative agents in humans. For example, 3-MeO-PCMe (Fig. 2b) was reported to be active at 11 mg (nasal insufflation of the HCl salt) with mild dissociative effects at this dose. Likewise, 3-Me-PCE, 3,4-MD-PCE, and 3,4-MD-PCPr (Fig. 2b) are potent dissociatives with activity beginning between 5 and 10 mg (nasal insufflation of HCl salts). 3,4-MD-PCPr was also reported to have about a 3.5 h duration with a 5 mg dose (personal communication).

7.3 Effects In Vivo

A number of *N*-alkylated arylcyclohexylamines were evaluated in a pigeon catalepsy test where catalepsy was considered present if the test animal was unable to right

itself when laid on their back (right reflex) and when the "head drop" was not observed. The minimum effective dose (MED) was defined as the lowest dose at which two-thirds of animals displayed catalepsy following intramuscular injection of test drugs. The results included MED values for 3-MeO-PCMe (>40 mg/kg), 3-MeO-PCE (10 mg/kg), 3-MeO-PCPr (20 mg/kg), 3-HO-PCMe (40 mg/kg), 3-HO-PCE (2.5 mg/kg), and 3-HO-PCPr (40 mg/kg). The MED determined for PCP was 2.5 mg/kg, which suggested that 3-HO-PCE was equipotent whereas 3-MeO-PCE dropped in potency by a factor of four (Reel et al. 1988). PCE, PCPr, and PCPy showed reduced potency relative to PCP in inducing a number of effects in dogs including tachycardia, mydriasis, analgesia, hypersecretion, hypernea, and flexor reflex depression. PCE and PCPr were about half and a third as potent as PCP in general PCP behavioral effects, respectively (Vaupel 1983). However, potencies maybe species and/or model specific as in Fischer-derived CDF male rats PCE and PCPr were 5.79 and 0.97 times as potent as PCP in a PCP discrimination paradigm (Shannon et al. 1981b).

7.4 Clinical Toxicology

Reports of acute toxicity associated with 3-MeO-PCE, 3-MeO-PCPr, and 3-HO-PCE could not be identified.

8 3-MeO-PCMo

3-MeO-PCMo was first offered as a research chemical in 2015 and appears to have been a novel compound at the time. Its origin as a research chemical was presumably meant to circumvent legislation in the UK. The aryl-unsubstituted parent compound PCMo was reported to be a "street analog" of PCP (Morris and Wallach 2014). Users have reported dissociative effects with 3-MeO-PCMo following parenteral and oral routes of administration. 3-MeO-PCMo is reported to have a rather low potency with active doses requiring 100–500 mg or greater to induce desired effects (Anonymous 2015a, b, c, 2018a). This makes it one of the least potent of the research chemical dissociatives. 3-MeO-PCMo was first notified to the EMCDDA in August 2015 (EMCDDA–Europol 2016). The syntheses and analytical characterizations of 3-MeO-PCMO and closely related analogs and positional isomers have been described (Ahmadi et al. 2011a, b; Colestock et al. 2018). The detection of a methylene linker analog called 3-MeO-PCMMo (Fig. 2) has been recently reported to the EMCDDA (EMCDDA–Europol 2017). Although information about this particular compound is currently unavailable, it has been shown that the *N*-methyl-1-(1-phenylcyclohexyl)methanamine structural template might open the door to a variety of interesting triple monoamine transporter blockers (e.g., Shao et al. 2011). The unsubstituted primary amine counterpart (1-phenylcyclohexyl)methanamine (PCMA) has been investigated and was described as having weak affinity for the PCP binding site ($K_i = 5{,}100$ nM, [^{3}H]-TCP in rat brain) along with anticonvulsant

effects (Thurkauf et al. 1990). The related isomer BnCP (Fig. 1) was a first generation illicit street analog of PCP.

8.1 Pharmacokinetics

Information about the pharmacokinetic properties of 3-MeO-PCMo could not be identified.

8.2 Pharmacodynamic Effects In Vitro

3-MeO-PCMo was found to have modest affinity for the PCP site of NMDAR with a K_i value of 252.9 nM ([^{3}H]MK-801, rat brain). This was slightly higher than ketamine (K_i = 323.9 nM) in this study but over tenfold lower than PCP (K_i = 22.1 nM) (Table 1). This modest NMDAR affinity is consistent with reports of its low potency in humans although it seems less potent than ketamine suggesting additional factors may be relevant. In addition to affinity for NMDAR, 3-MeO-PCMo showed modest affinity at SERT (K_i = 697 nM), sigma-2 (K_i = 299 nM), and alpha-2A (K_i = 1,446 nM), but lacked affinity (IC_{50} > 10,000 nM) for DAT, NET, MOR, sigma-1, and many other CNS receptor sites (Colestock et al. 2018). A number of related morpholine-containing arylcyclohexylamines were also evaluated in this study, which included 2-MeO-PCMo, 4-MeO-PCMo, 3-Me-PCMo, 3,4-PCMo, and PCMo (Table 1). 3-MeO-PCMo had a slightly higher NMDAR affinity than PCMo. Of the three methoxy-substituted PCMo positional isomers, the rank order of affinity for the PCP site of NMDAR was 3-, 2-, and 4-MeO-PCMo (Table 1) (Colestock et al. 2018), again consistent with other arylcyclohexylamines (Wallach 2014). Of the series of PCMo compounds investigated, only 3-Me-PCMo had higher NMDAR affinity than 3-MeO-PCMo. The high affinity seen with 3-Me-PCMo and 3-MeO-PCMo, compared to PCMo, is again in agreement with known SAR where electron-donating groups located at the 3-position increase or do not affect NMDAR affinity. All investigated PCMo analogs showed affinity for sigma-2 but not sigma-1 with K_i values for sigma-2 ranging between 167 nM (PCMo) and 808 nM (4-MeO-PCMo). PCMo was the only compound in the series that showed weak affinity at the serotonin 5-HT_{2A} receptor (K_i = 3,639 nM), and 3-Me-PCMo was the only compound with affinity toward the alpha-2C adrenergic receptor subtype (K_i = 1,448 nM) (Colestock et al. 2018).

8.3 Effects In Vivo

PCMo and several aryl-substituted derivatives have shown analgesic effects in rats using tail immersion and formalin tests (Ahmadi et al. 2011a, b), but specific information about 3-MeO-PCMo could not be identified. PCMo showed

significantly reduced potency relative to PCP in inducing a number of effects in dogs including tachycardia, mydriasis, analgesia, hypersecretion, hypernea, and flexor reflex depression. Similar to 3-MeO-PCMo, PCMo has been reported to have low potency in humans (Morris and Wallach 2014). This is confirmed in animal studies; for example, PCMo was about 1/40th as potent as PCP in inducing general PCP-like behavioral effects in dogs (Vaupel 1983), and PCMo was 1/10th the potency of PCP in a PCP discrimination paradigm in rats which was comparable to ketamine (Shannon et al. 1981b).

8.4 Clinical Toxicology

Reports of acute toxicity associated with 3-MeO-PCMo could not be identified.

9 Conclusions

Scientific and psychonautic curiosity and the motivation to search for drugs perceived to be potentially useful for self-medication appeared to have played a role in establishing collaborations between members of online drug forums. These collaborations provided ideas for compounds that subsequently appeared on the research chemical market. The available pharmacological data suggest that the phencyclidine-based NPS showed high to moderate affinities for the NMDAR, which seemed to correlate with the dissociative activity in humans. The comparison between available information on 3-MeO-PCP and 4-MeO-PCP has consistency with results from early studies where *meta*-substitution on the phenyl ring with an electron-donating group often leads to increased or equipotent NMDAR affinity whereas *para*- substitution decrease affinity. Correspondingly, 3-MeO-PCP is reported to be at least an order of magnitude more potent than 4-MeO-PCP in humans. Likewise, switching the piperidine ring of PCP to a morpholine as in PCMo leads to a loss of NMDAR affinity and potency in humans. Some non-NMDAR receptor interactions have been noted with the arylcyclohexylamines described including affinity to monoamine transporters, sigma receptors, and occasionally also opioid receptor and alpha-adrenergic subtypes. Clinical features reported from acute intoxication cases have included confusion, hallucination, dissociation, catatonia, euphoria, comatose states, nystagmus, as well as hypertension and tachycardia. The research chemical market is constantly evolving in response to numerous factors including legislative and market demand. One may thus anticipate a variety of new dissociative substances to emerge. The employment of chemical manufacturers overseas makes it a relatively straightforward proposition for entrepreneurs in the NPS field to manifest ideas for new compounds. The potential for some of these compounds to have therapeutic uses is captivating. More research is warranted to further explore the properties of these dissociative drugs, particularly their potential medical value, and this field is not anticipated to get boring any time soon.

References

Aguayo LG, Albuquerque EX (1986) Effects of phencyclidine and its analogs on the end-plate current of the neuromuscular junction. J Pharmacol Exp Ther 239:15–24

Ahmadi A, Khalili M, Hajikhani R, Naserbakht M (2011a) New morpholine analogues of phencyclidine: chemical synthesis and pain perception in rats. Pharmacol Biochem Behav 98:227–233

Ahmadi A, Khalili M, Hajikhani R, Naserbakht M (2011b) Synthesis and determination of acute and chronic pain activities of 1-[1-(4-methylphenyl) (cyclohexyl)] morpholine as a new phencyclidine derivative in rats. Arzneimittelforschung 61:92–97

Allard S, Deslandes G, Gaborit B, Lomenech H, Pineau A, Jolliet P, Garret C, Monteil-Ganiere C (2017) 3-MeO-PCP et 4-MeO-PCP: confusion des isomères et risque majeur de toxicité. Toxicol Anal Clin 29:S47–S48

Aniline O, Pitts FN Jr (1982) Phencyclidine (PCP): a review and perspectives. Crit Rev Toxicol 10:145–177

Anonymous (1960) Cyclohexylamine compounds and methods for producing the same. Patent no. GB851782. Parke-Davis, Detroit

Anonymous (2008) Bluelight. Thread: the Big & Dandy 4-MeO-PCP thread. http://bluelight.org/vb/threads/391328-The-Big-amp-Dandy-4-MeO-PCP-Thread. Accessed 3 Feb 2018

Anonymous (2012a) Bluelight. Psychedelic drugs. Thread: 3-ho-pcp. http://www.bluelight.org/vb/threads/623701-3-ho-pcp. Accessed 3 Feb 2018

Anonymous (2012b) Bluelight. Trip reports. Thread: a trial with 3-OH-PCP. http://www.bluelight.org/vb/threads/633737-3-HO-PCE-fumarate. Accessed 3 Feb 2018

Anonymous (2015a) 3-MeO-PCP's little brother: an experience with 3-MeO-PCMo (exp106299). Erowid.org. 28 Aug 2015. https://erowid.org/experiences/exp_pdf.php?ID=106299&format=pdf. Accessed 3 Feb 2018

Anonymous (2015b) A novel dissociative but not a novel feeling: an experience with 3-MeO-PCMo (exp106459). Erowid.org. 28 Aug 2015. https://erowid.org/experiences/exp_pdf.php?ID=106459&format=pdf. Accessed 3 Feb 2018

Anonymous (2015c) UK chemical research, chemical discussion, psychedelics, 3-MeO-PCMo. https://www.ukchemicalresearch.org/Thread-3-MeO-PCMo. Accessed 3 Feb 2018

Anonymous (2017) Bluelight. Psychedelic drugs. Thread: the big & Dandy 3-HO-PCE thread. http://bluelight.org/vb/threads/812314-The-Big-amp-Dandy-3-HO-PCE-Thread. Accessed 3 Feb 2018

Anonymous (2018a) Bluelight psychedelic drugs index. Thread. Novel dissociative 3-MeO-PCMo. http://www.bluelight.org/vb/threads/758083-Novel-dissociative-3-MeO-PCMo. Accessed 3 Feb 2018

Anonymous (2018b) Erowid experience vaults report Id: 110467. Accessed 3 Feb 2018

Backberg M, Beck O, Helander A (2015) Phencyclidine analog use in Sweden–intoxication cases involving 3-MeO-PCP and 4-MeO-PCP from the STRIDA project. Clin Toxicol (Phila) 53:856–864

Bakota E, Arndt C, Romoser AA, Wilson SK (2016) Fatal intoxication involving 3-MeO-PCP: a case report and validated method. J Anal Toxicol 40:504–510

Balster RL, Chait LD (1976) The behavioral pharmacology of phencyclidine. Clin Toxicol (Phila) 9:513–528

Balster RL, Woolverton WL (1980) Continuous-access phencyclidine self-administration by rhesus monkeys leading to physical dependence. Psychopharmacology 70:5–10

Barceloux DG (2012) Phencyclidine and phencyclidine analogues. In: Barceloux DG (ed) Medical toxicology of drugs abuse: synthesized chemicals and psychoactive plants. Wiley, Hoboken, pp 608–632

Baselt RC (2011) Disposition of toxic drugs and chemicals in man, 9th edn. Biomedical Publications, Seal Beach, pp 1323–1325

Bertol E, Pascali J, Palumbo D, Catalani V, Di Milia MG, Fioravanti A, Mari F, Vaiano F (2017) 3-MeO-PCP intoxication in two young men: first in vivo detection in Italy. Forensic Sci Int 274:7–12

Bey T, Patel A (2007) Phencyclidine intoxication and adverse effects: a clinical and pharmacological review of an illicit drug. Cal J Emerg Med 8:9–14

Boldyrev AA, Carpenter DO, Johnson P (2005) Emerging evidence for a similar role of glutamate receptors in the nervous and immune systems. J Neurochem 95:913–918

Brady KT, Balster RL (1981) Discriminative stimulus properties of phencyclidine and five analogues in the squirrel monkey. Pharmacol Biochem Behav 14:213–218

Brine GA, Boldt KG, Coleman ML, Carroll FI (1984) Carbon-13 nuclear magnetic resonance spectra of phencyclidine analogs substituted in the piperidine and aromatic rings. J Heterocycl Chem 21:71–75

Burns RS, Lerner SE (1976) Perspectives: acute phencyclidine intoxication. Clin Toxicol 9:477–501

Cadinu D, Grayson B, Podda G, Harte MK, Doostdar N, Neill JC (2017) NMDA receptor antagonist rodent models for cognition in schizophrenia and identification of novel drug treatments, an update. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2017.1011.1045

Carroll FI, Brine GA, Boldt KG, Mascarella SW, Moreland CG, Sumner SJ, Burgess JP, Stejskal EO (1988) Solid state conformation of phencyclidine and phencyclidine analogs. In: Domino EF, Kamenka JM (eds) Sigma and phencyclidine-like compounds as molecular probes in biology. NPP Books, Ann Arbor, pp 91–106

Casale JF (2011) 4-Methoxyphencyclidine: an analytical profile. Microgram J 8:39–42

Chang BN, Smith MP (2017) A case of unusual drug screening results. Clin Chem 63:958–961

Chaudieu I, Vignon J, Chicheportiche M, El Harfi A, Kamenka JM, Chicheportiche R (1987) Comparaison entre les sites de fixation de la [^{3}H]phencyclidine (PCP) et de la [^{3}H](thiényl-2)-1 cyclohexylpipéridine (TCP) dans le système nerveux central de rat. Eur J Med Chem 22:359–362

Chaudieu I, Vignon J, Chicheportiche M, Kamenka JM, Trouiller G, Chicheportiche R (1989) Role of the aromatic group in the inhibition of phencyclidine binding and dopamine uptake by PCP analogs. Pharmacol Biochem Behav 32:699–705

Chen G (1981) The neuropharmacology of phencyclidine. In: Domino EF (ed) PCP (phencyclidine): historical and current perspectives. NPP Books, Ann Arbor, pp 9–23

Cho AK, Hallström G, Matsumotto RM, Kammerer RC (1983) The metabolism of the piperidine ring of phencyclidine. In: Kamenka JM, Domino EF, Geneste P (eds) Phencyclidine and related arylcyclohexylamines: present and future applications. NPP Books, Ann Arbor, pp 205–214

Cho AK, Hiramatsu M, Schmitz DA, Nabeshima T, Kameyama T (1991) Pharmacokinetic and pharmacodynamic properties of some phencyclidine analogs in rats. Pharmacol Biochem Behav 39:947–953

Clouet DH (1986) Phencyclidine: an update, NIDA research monograph, vol 64. United States Department of Health and Human Services, National Institute on Drug Abuse, Rockville

Colestock T, Wallach J, Mansi M, Filemban N, Morris H, Elliott SP, Westphal F, Brandt SD, Adejare A (2018) Syntheses, analytical and pharmacological characterizations of the 'legal high' 4-[1-(3-methoxyphenyl)cyclohexyl]morpholine (3-MeO-PCMo) and analogues. Drug Test Anal 10:272–283

Cone EJ, McQuinn RL, Shannon HE (1984) Structure-activity relationship studies of phencyclidine derivatives in rats. J Pharmacol Exp Ther 228:147–153

Cook CE, Brine DR, Jeffcoat AR, Hill JM, Wall ME, Perez-Reyes M, Di Guiseppi SR (1982) Phencyclidine disposition after intravenous and oral doses. Clin Pharmacol Ther 31:625–634

Coppola M, Mondola R (2013) Is methoxydine a new rapid acting antidepressant for the treatment of depression in alcoholics? Med Hypotheses 81:10–14

Costa JF, Speaker TJ (1983) Chromatographic and spectral properties of some aryl-substituted phencyclidine analogs. J Anal Toxicol 7:252–256

Cravey RH, Reed D, Ragle JL (1979) Phencyclidine-related deaths: a report of nine fatal cases. J Anal Toxicol 3:199–201

Cull-Candy S, Brickley S, Farrant M (2001) NMDA receptor subunits: diversity, development and disease. Curr Opin Neurobiol 11:327–335

De Paoli G, Brandt SD, Wallach J, Archer RP, Pounder DJ (2013) From the street to the laboratory: analytical profiles of methoxetamine, 3-methoxyeticyclidine and 3-methoxyphencyclidine and their determination in three biological matrices. J Anal Toxicol 37:277–283

Domino EF (1964) Neurobiology of phencyclidine (Sernyl), a drug with an unusual spectrum of pharmacological activity. Int Rev Neurobiol 6:303–347

Domino EF (ed) (1981) PCP (phencyclidine): historical and current perspectives. NPP Books, Ann Arbor

Domino EF (2010) Taming the ketamine tiger. Anesthesiology 113:678–684

Domino EF, Kamenka JM (eds) (1988) Sigma and phencyclidine-like compounds as molecular probes in biology. NPP Books, Ann Arbor

Domino EF, Rocki W, Holsztynska EJ, Lee S (1983) Structure-activity relationships of phencyclidine derivatives and related substances on mouse motor performance. In: Kamenka JM, Domino EF, Geneste P (eds) Phencyclidine and related arylcyclohexylamines: present and future applications. NPP Books, Ann Arbor, pp 397–409

Done AK, Aronow R, Miceli JN (1978) The pharmacokinetics of phencyclidine in overdosage and its treatment. In: Petersen RC, Stillman RC (eds) Phencyclidine (PCP) abuse: an appraisal, NIDA research monograph, vol 21. United States Department of Health and Human Services, National Institute on Drug Abuse, Rockville, pp 210–217

Dresen S, Blake D, Taylor A, Williams K (2014) Identification and quantitation of designer drugs in urine by LC-MS/MS. Technical note, AB SCIEX, Framingham. https://sciex.com.cn/Documents/Applications/RUO-MKT-02-1407-A_technote_designer_drugs.pdf. Accessed 3 Feb 2018

Driscoll JP, Kornecki K, Wolkowski JP, Chupak L, Kalgutkar AS, O'Donnell JP (2007) Bioactivation of phencyclidine in rat and human liver microsomes and recombinant P450 2B enzymes: evidence for the formation of a novel quinone methide intermediate. Chem Res Toxicol 20:1488–1497

El M'Barki C, Elhallaqui M (2017) Quantum calculations to construct a 3D-QSAR model based on PCP-TCP derivatives and molecular docking with NMDA receptor. J Mater Environ Sci 8:1391–1400

EMCDDA–Europol (2011) EMCDDA–Europol 2010 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/644/EMCDDA-Europol_Annual_Report_2010A_281336.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2012) EMCDDA–Europol 2011 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/689/EMCDDA-Europol_Annual_Report_2011_2012_final_335568.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2013) New drugs in Europe, 2012. EMCDDA–Europol 2012 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/734/EMCDDA-Europol_2012_Annual_Report_final_439477.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2015) EMCDDA–Europol 2014 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/1018/TDAN15001ENN.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2016) EMCDDA–Europol 2015 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/2880/TDAS16001ENN.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2017) EMCDDA–Europol 2016 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/4724/TDAN17001ENN_PDFWEB.pdf. Accessed 3 Feb 2018

Feldman HW, Agar MH, Beschner GM (eds) (1979) Angel dust. An ethnographic study of phencyclidine users. Lexington Books, Lexington

Frohlich J, Van Horn JD (2014) Reviewing the ketamine model for schizophrenia. J Psychopharmacol 28:287–302

Gable MS, Gavali S, Radner A, Tilley DH, Lee B, Dyner L, Collins A, Dengel A, Dalmau J, Glaser CA (2009) Anti-NMDA receptor encephalitis: report of ten cases and comparison with viral encephalitis. Eur J Clin Microbiol Infect Dis 28:1421–1429

Geneste P, Kamenka JM, Ung SN, Herrmann P, Goudal R, Trouiller G (1979) Determination conformationelle de dérivés de la phencyclidine en vue d'une correlation structure-activité. Eur J Med Chem Chim Ther 14:301–308

Giannini AJ, Castellani S (1982) A case of phenylcyclohexylpyrrolidine (PHP) intoxication treated with physostigmine. J Toxicol Clin Toxicol 19:505–508

Giannini AJ, Price WA, Loiselle RH, Malone DW (1985) Treatment of phenylcyclohexylpyrrolidine (PHP) psychosis with haloperidol. J Toxicol Clin Toxicol 23:185–189

Gintzler AR, Zukin RS, Zukin SR (1982) Effects of phencyclidine and its derivatives on enteric neurones. Br J Pharmacol 75:261–267

Giros B, El Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T, Caron MG (1992) Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. Mol Pharmacol 42:383–390

Gole DJ, Pirat JL, Domino EF (1988a) New aspects of phencyclidine (PCP) metabolism. In: Domino EF, Kamenka JM (eds) Sigma and phencyclidine-like compounds as molecular probes in biology. NPP Books, Ann Arbor, pp 625–641

Gole DJ, Pirat JL, Kamenka JM, Domino EF (1988b) Hydroxy metabolites of phencyclidine. Identification and quantitation of two novel metabolites. Drug Metab Dispos 16:386–391

Gole DJ, Pirat JL, Kamenka JM, Kalir A, Domino EF (1988c) Analysis of phencyclidine (PCP) and other structurally related compounds by gas chromatography-mass spectrometry (GC-MS). In: Domino EF, Kamenka JM (eds) Sigma and phencyclidine-like compounds as molecular probes in biology. NPP Books, Ann Arbor, pp 79–89

Greifenstein FE, Devault M, Yoshitake J, Gajewski JE (1958) A study of a 1-aryl cyclo hexyl amine for anesthesia. Anesth Analg 37:283–294

Hallstrom G, Kammerer RC, Nguyen CH, Schmitz DA, Di Stefano EW, Cho AK (1983) Phencyclidine metabolism in vitro. The formation of a carbinolamine and its metabolites by rabbit liver preparations. Drug Metab Dispos 11:47–53

Hamon J, Vignon J, Kamenka JM (1996) Effect of lowered lipophilicity on the affinity of PCP analogs for the PCP receptor and the dopamine transporter. Eur J Med Chem 31:489–495

Haradahira T, Sasaki S, Maeda M, Kobayashi K, Inoue O, Tomita U, Nishikawa T, Suzuki K (1998) Synthesis and brain distribution of carbon-11 labeled analogs of antagonists for the NMDA receptor coupled PCP-binding site. J Label Compd Radiopharm 41:843–858

Hardingham GE, Bading H (2003) The Yin and Yang of NMDA receptor signalling. Trends Neurosci 26:81–89

Haring R, Theomy S, Kalir A, Sokolovsky M (1983) Characterization of the interaction of phencyclidine and its derivatives with the ionic channel of the nicotinic receptor. In: Chambers CM, Chambers PL, Gitter S (eds) Toxicology in the use, misuse, and abuse of food, drugs, and chemicals, Archives of Toxicology (supplement), vol 6. Springer, Berlin, pp 81–90

Hearne E, Van Hout MC (2016) "Trip-sitting" in the black hole: a netnographic study of dissociation and indigenous harm reduction. J Psychoactive Drugs 48:233–242

Holsztynska EJ, Domino EF (1983) Overview of phencyclidine biotransformation. In: Kamenka JM, Domino EF, Geneste P (eds) Phencyclidine and related arylcyclohexylamines: present and future applications. NPP Books, Ann Arbor, pp 157–194

Holsztynska EJ, Domino EF (1985) Biotransformation of phencyclidine. Drug Metab Rev 16:285–320

Hori T, Suzuki T, Baba A, Abe S, Yamamoto T, Moroji T, Shiraishi H (1996) Effects of phencyclidine metabolites on serotonin uptake in rat brain. Neurosci Lett 209:153–156

Hundt W, Danysz W, Holter SM, Spanagel R (1998) Ethanol and N-methyl-D-aspartate receptor complex interactions: a detailed drug discrimination study in the rat. Psychopharmacology (Berl) 135:44–51

Itzhak Y (1988) Pharmacological specificity of some psychotomimetic and antipsychotic agents for the sigma and PCP binding sites. Life Sci 42:745–752

Itzhak Y, Kalir A, Sarne Y (1981a) On the opioid nature of phencyclidine and its 3-hydroxy derivative. Eur J Pharmacol 73:229–233

Itzhak Y, Kalir A, Weissman BA, Cohen S (1981b) Receptor binding and antinociceptive properties of phencyclidine opiate-like derivatives. Eur J Pharmacol 72:305–311

Jackson JE (1989) Phencyclidine pharmacokinetics after a massive overdose. Ann Intern Med 111:613–615

Javitt DC (2007) Glutamate and schizophrenia: phencyclidine, N-methyl-D-aspartate receptors, and dopamine-glutamate interactions. Int Rev Neurobiol 78:69–108

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301–1308

Johansson A, Lindstedt D, Roman M, Thelander G, Nielsen EI, Lennborn U, Sandler H, Rubertsson S, Ahlner J, Kronstrand R, Kugelberg FC (2017) A non-fatal intoxication and seven deaths involving the dissociative drug 3-MeO-PCP. Forensic Sci Int 275:76–82

Johnson N, Itzhak Y, Pasternak GW (1984) Interaction of two phencyclidine opiate-like derivatives with ^{3}H-opioid binding sites. Eur J Pharmacol 101:281–284

Jordan S, Chen R, Fernalld R, Johnson J, Regardie K, Kambayashi J, Tadori Y, Kitagawa H, Kikuchi T (2006) In vitro biochemical evidence that the psychotomimetics phencyclidine, ketamine and dizocilpine (MK-801) are inactive at cloned human and rat dopamine D_2 receptors. Eur J Pharmacol 540:53–56

Kalir A (1981) Structure activity relationships of phencyclidine derivatives. In: Domino EF (ed) PCP (phencyclidine), historical and current perspectives. NPP Books, Ann Arbor, pp 31–46

Kalir A, Edery H, Pelah Z, Balderman D, Porath G (1969) 1-Phenylcycloalkylamine derivatives. II. Synthesis and pharmacological activity. J Med Chem 12:473–477

Kalir A, Elkavets R, Pri-Bar I, Buchman O (1978) Structure-activity relationship of some phencyclidine derivatives: in vivo studies in mice. Eur J Med Chem Chim Ther 13:17–24

Kamenka JM, Geneste P (1981) Synthesis, conformation, and physical properties of phencyclidine and its derivatives. In: Domino EF (ed) PCP (phencyclidine): historical and current perspectives. NPP Books, Ann Arbor, pp 47–82

Kamenka JM, Geneste P (1983) The biologically active conformation of PCP and its consequences: a hypothesis. In: Kamenka JM, Domino EF, Geneste P (eds) Phencyclidine and related arylcyclohexylamines: present and future applications. NPP Books, Ann Arbor, pp 1–12

Kamenka JM, Chiche B, Goudal R, Geneste P, Vignon J, Vincent JP, Lazdunski M (1982) Chemical synthesis and molecular pharmacology of hydroxylated 1-(1-phenylcyclohexyl-piperidine derivatives. J Med Chem 25:431–435

Kammerer RC, Schmitz DA, Hwa JJ, Cho AK (1984) Induction of phencyclidine metabolism by phencyclidine, ketamine, ethanol, phenobarbital and isosafrole. Biochem Pharmacol 33:599–604

Kapur S, Seeman P (2002) NMDA receptor antagonists ketamine and PCP have direct effects on the dopamine D_2 and serotonin 5-HT_2 receptors-implications for models of schizophrenia. Mol Psychiatry 7:837–844

Karakas E, Furukawa H (2014) Crystal structure of a heterotetrameric NMDA receptor ion channel. Science 344:992–997

Kemp JA, McKernan RM (2002) NMDA receptor pathways as drug targets. Nat Neurosci 5(Suppl):1039–1042

Kozlowski MR, Browne RG, Vinick FJ (1986) Discriminative stimulus properties of phencyclidine (PCP)-related compounds: correlations with ^{3}H-PCP binding potency measured autoradiographically. Pharmacol Biochem Behav 25:1051–1058

Krotulski AJ, Papsun DM, Friscia M, Swartz JL, Holsey BD, Logan BK (2017) Fatality following ingestion of tetrahydrofuranylfentanyl, U-49900 and methoxy-phencyclidine. J Anal Toxicol 42:e27–e32

Krystal JH, Petrakis IL, Webb E, Cooney NL, Karper LP, Namanworth S, Stetson P, Trevisan LA, Charney DS (1998) Dose-related ethanol-like effects of the NMDA antagonist, ketamine, in recently detoxified alcoholics. Arch Gen Psychiatry 55:354–360

Krystal JH, Petrakis IL, Krupitsky E, Schutz C, Trevisan L, D'Souza DC (2003) NMDA receptor antagonism and the ethanol intoxication signal: from alcoholism risk to pharmacotherapy. Ann N Y Acad Sci 1003:176–184

Largent BL, Gundlach AL, Snyder SH (1986) Pharmacological and autoradiographic discrimination of sigma and phencyclidine receptor binding sites in brain with (+)-[^{3}H]SKF 10,047, (+)-[^{3}H]-3-[3-hydroxyphenyl]-N-(1-propyl)piperidine and [^{3}H]-1-[1-(2-thienyl) cyclohexyl]piperidine. J Pharmacol Exp Ther 238:739–748

Leander JD, Rathbun RC, Zimmerman DM (1988) Anticonvulsant effects of phencyclidine-like drugs: relation to N-methyl-D-aspartic acid antagonism. Brain Res 454:368–372

Leccese AP, Marquis KL, Mattia A, Moreton JE (1986) The convulsant and anticonvulsant effects of phencyclidine (PCP) and PCP analogues in the rat. Behav Brain Res 19:163–169

Lee CH, Lu W, Michel JC, Goehring A, Du J, Song X, Gouaux E (2014) NMDA receptor structures reveal subunit arrangement and pore architecture. Nature 511:191–197

Lehmann S, Kieliba T, Beike J, Thevis M, Mercer-Chalmers-Bender K (2017) Determination of 74 new psychoactive substances in serum using automated in-line solid-phase extraction-liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 1064:124–138

Lipton SA (2006) Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. Nat Rev Drug Discov 5:160–170

Lodge D, Mercier MS (2015) Ketamine and phencyclidine: the good, the bad and the unexpected. Br J Pharmacol 172:4254–4276

Lomenech H, Schreck B, Caillet P, Ganiere C, Allard S, Jolliet P, Victorri-Vigneau C (2017) Coma following AB-FUBINACA and 3-MeO-PCP consumption: a case report [poster abstract]. Fundam Clin Pharmacol 31:S55

Lü W et al (2017) Cryo-EM structures of the triheteromeric NMDA receptor and its allosteric modulation. Science 355(6331):eaal3729

Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R (1959) Study of a new schizophrenomimetic drug; sernyl. AMA Arch Neurol Psychiatry 81:363–369

Maayani S, Weinstein H, Ben-zvi N, Cohen S, Sokolovsky M (1974) Psychotomimetics as anticholinergic agents. I. 1-Cyclohexylpiperidine derivatives. Anticholinesterase activity and antagonistic activity to acetylcholine. Biochem Pharmacol 23:1263–1281

Maddox VH, Godefroi EF, Parcell RF (1965) The synthesis of phencyclidine and other 1-arylcyclohexylamines. J Med Chem 8:230–235

Marquis KL, Moreton JE (1987) Animal models of intravenous phencyclinoid self-administration. Pharmacol Biochem Behav 27:385–389

McCarron MM, Schulze BW, Thompson GA, Conder MC, Goetz WA (1981) Acute phencyclidine intoxication: incidence of clinical findings in 1,000 cases. Ann Emerg Med 10:237–242

McIntyre IM, Trochta A, Gary RD, Storey A, Corneal J, Schaber B (2015) A fatality related to two novel hallucinogenic compounds: 4-methoxyphencyclidine and 4-hydroxy-N-methyl-N-ethyltryptamine. J Anal Toxicol 39:751–755

McMillan DE, Evans EB, Wessinger WD, Owens SM (1988) Structure-activity relationships of arylcyclohexylamines as discriminative stimuli in pigeons. J Pharmacol Exp Ther 247:1086–1092

Mendelsohn LG, Kerchner GA, Kalra V, Zimmerman DM, Leander JD (1984) Phencyclidine receptors in rat brain cortex. Biochem Pharmacol 33:3529–3535

Meyer JS, Greifenstein F, Devault M (1959) A new drug causing symptoms of sensory deprivation. Neurological, electroencephalographic and pharmacological effects of Sernyl. J Nerv Ment Dis 129:54–61

Michely JA, Manier SK, Caspar AT, Brandt SD, Wallach J, Maurer HH (2017) New psychoactive substances 3-methoxyphencyclidine (3-MeO-PCP) and 3-methoxyrolicyclidine (3-MeO-PCPy): metabolic fate elucidated with rat urine and human liver preparations and their detectability in urine by GC-MS, "LC-(high resolution)-MS^n" and "LC-(high resolution)-MS/MS". Curr Neuropharmacol 15:692–712

Misselbrook GP, Hamilton EJ (2012) Out with the old, in with the new? Case reports of the clinical features and acute management of two novel designer drugs. Acute Med 11:157–160

Mitchell-Mata C, Thomas B, Peterson B, Couper F (2017) Two fatal intoxications involving 3-methoxyphencyclidine. J Anal Toxicol 41:503–507

Morris H, Wallach J (2014) From PCP to MXE: a comprehensive review of the non-medical use of dissociative drugs. Drug Test Anal 6:614–632

Murray JB (2002) Phencyclidine (PCP): a dangerous drug, but useful in schizophrenia research. J Psychol 136:319–327

Nishimura M, Sato K, Okada T, Yoshiya I, Schloss P, Shimada S, Tohyama M (1998) Ketamine inhibits monoamine transporters expressed in human embryonic kidney 293 cells. Anesthesiology 88:768–774

Ogden KK, Traynelis SF (2011) New advances in NMDA receptor pharmacology. Trends Pharmacol Sci 32:726–733

Ohta S, Masumoto H, Takeuchi K, Hirobe M (1987) A phenolic metabolite of phencyclidine: the formation of a pharmacologically active metabolite by rat liver microsomes. Drug Metab Dispos 15:583–584

Paoletti P, Bellone C, Zhou Q (2013) NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci 14:383–400

Petersen RC, Stillman RC (1978) Phencyclidine (PCP) abuse: an appraisal, NIDA research monograph, vol 21. United States Department of Health and Human Services, National Institute on Drug Abuse, Rockville

Pollard JC, Uhr L, Stern E (1965) Drugs and phantasy: the effects of LSD, psilocybin, and Sernyl on college students. Little, Brown, Boston

Ponchant M, Dreux Y, Kamenka JM, Chicheportiche R, Beaucourt JP (1990) Synthesis of iodine-125-labeled 3-[^{125}I]-iodophencyclidine for biological studies. J Label Compd Radiopharm 28:1059–1064

Pradhan SN (1984) Phencyclidine (PCP): some human studies. Neurosci Biobehav Rev 8:493–501

Quirion R, Hammer RP Jr, Herkenham M, Pert CB (1981) Phencyclidine (angel dust)/sigma "opiate" receptor: visualization by tritium-sensitive film. Proc Natl Acad Sci U S A 78:5881–5885

Reel JK, Leander JD, Mendelsohn LG, Schoepp DD, Ornstein PL, Evrard DA, Hermann RB, Zimmerman DM (1988) The search for a PCP antagonist: synthesis and characterization of novel arylcyclohexylamine derivatives. In: Domino EF, Kamenka JM (eds) Sigma and phencyclidine-like compounds as molecular probes in biology. NPP Books, Ann Arbor, pp 27–33

Risner ME (1982) Intravenous self-administration of phencyclidine and related compounds in the dog. J Pharmacol Exp Ther 221:637–644

Rogawski MA, Thurkauf A, Yamaguchi S, Rice KC, Jacobson AE, Mattson MV (1989) Anticonvulsant activities of 1-phenylcyclohexylamine and its conformationally restricted analog 1,1-pentamethylenetetrahydroisoquinoline. J Pharmacol Exp Ther 249:708–712

Roth BL, Gibbons S, Arunotayanun W, Huang XP, Setola V, Treble R, Iversen L (2013) The ketamine analogue methoxetamine and 3- and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor. PLoS One 8:e59334

Roth BL, Gibbons S, Arunotayanun W, Huang XP, Setola V, Treble R, Iversen L (2018) Correction: the ketamine analogue methoxetamine and 3- and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor. PLoS One 13: e0194984

Seeman P, Ko F, Tallerico T (2005) Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics. Mol Psychiatry 10:877–883

Shannon HE (1981a) Discriminative stimulus effects of phencyclidine: structure-activity relationships. In: Domino EF (ed) PCP (phencyclidine): historical and current perspectives. NPP Books, Ann Arbor, pp 311–335

Shannon HE (1981b) Evaluation of phencyclidine analogs on the basis of their discriminative stimulus properties in the rat. J Pharmacol Exp Ther 216:543–551

Shannon HE, DeGregorio CM (1981) Effects of N-substituted analogs and metabolites of phencyclidine on avoidance behavior in the rat. J Pharmacol Exp Ther 218:55–62

Shao L, Hewitt MC, Wang F, Malcolm SC, Ma J, Campbell JE, Campbell UC, Engel SR, Spicer NA, Hardy LW, Schreiber R, Spear KL, Varney MA (2011) Discovery of N-methyl-1-(1-phenylcyclohexyl)methanamine, a novel triple serotonin, norepinephrine, and dopamine reuptake inhibitor. Bioorg Med Chem Lett 21:1438–1441

Showalter CV, Thornton WE (1977) Clinical pharmacology of phencyclidine toxicity. Am J Psychiatry 134:1234–1238

Shulgin AT, MacLean DE (1976) Illicit synthesis of phencyclidine (PCP) and several of its analogs. Clin Toxicol 9:553–560

Siegel RK (1978) Phencyclidine and ketamine intoxication: a study of four populations of recreational users. In: Petersen RC, Stillman RC (eds) Phencyclidine (PCP) abuse: an appraisal, NIDA research monograph, vol 21. United States Department of Health and Human Services, National Institute on Drug Abuse, Rockville, pp 119–147

Smith RC, Meltzer HY, Arora RC, Davis JM (1977) Effects of phencyclidine on [^{3}H]catecholamine and [^{3}H]serotonin uptake in synaptosomal preparations from rat brain. Biochem Pharmacol 26:1435–1439

Stefek M, Ransom RW, DiStefano EW, Cho AK (1990) The alpha carbon oxidation of some phencyclidine analogues by rat tissue and its pharmacological implications. Xenobiotica 20:591–600

Steinpreis RE (1996) The behavioral and neurochemical effects of phencyclidine in humans and animals: some implications for modeling psychosis. Behav Brain Res 74:45–55

Stevenson R, Tuddenham L (2014) Novel psychoactive substance intoxication resulting in attempted murder. J Forensic Legal Med 25:60–61

Stillman R, Petersen RC (1979) The paradox of phencyclidine (PCP) abuse. Ann Intern Med 90:428–430

Suzuki T, Yamamoto T, Hori T, Baba A, Shiraishi H, Ito T, Piletz JE, Ho IK (1996) Autoradiographic study on the pharmacological characteristics of [^{3}H]3-OH-PCP binding sites in rat brain. Eur J Pharmacol 310:243–255

Thornton S, Lisbon D, Lin T, Gerona R (2017) Beyond ketamine and phencyclidine: analytically confirmed use of multiple novel arylcyclohexylamines. J Psychoactive Drugs 49:289–293

Thurkauf A, de Costa B, Yamaguchi S, Mattson MV, Jacobson AE, Rice KC, Rogawski MA (1990) Synthesis and anticonvulsant activity of 1-phenylcyclohexylamine analogues. J Med Chem 33:1452–1458

Tikhonova IG, Baskin II, Palyulin VA, Zefirov NS (2004) 3D-model of the ion channel of NMDA receptor: qualitative and quantitative modeling of the blocker binding. Dokl Biochem Biophys 396:181–186

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R (2010) Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev 62:405–496

Vaupel DB (1983) Phencyclidine studies in the dog. Pharmacology of PCP, profiles of eight analogues abused by man and the similarity of PCP to the opioid NANM (N-allylnormetazocine or SKF 10,047). In: Domino EF (ed) Phencyclidine and related arylcyclohexylamines: present and future applications. NPP Books, Ann Arbor, pp 347–367

Vaupel DB, McCoun D, Cone EJ (1984) Phencyclidine analogs and precursors: rotarod and lethal dose studies in the mouse. J Pharmacol Exp Ther 230:20–27

Vignon J, Vincent JP, Bidard JN, Kamenka JM, Geneste P, Monier S, Lazdunski M (1982) Biochemical properties of the brain phencyclidine receptor. Eur J Pharmacol 81:531–542

Vignon J, Cerruti C, Chaudieu I, Pinet V, Chicheportiche M, Kamenka JM, Chicheportiche R (1988) Interaction of molecules in the phencyclidine series with the dopamine uptake system: correlation with their binding properties to the phencyclidine receptor. Binding properties of [^{3}H]BTCP, a new PCP analog, to the dopamine uptake complex. In: Domino EF, Kamenka JM (eds) Sigma and phencyclidine-like compounds as molecular probes in biology. NPP Books, Ann Arbor, pp 199–208

Walberg CB, McCarron MM, Schulze BN (1983) Quantitation of phencyclidine in serum by enzyme immunoassay: results in 405 patients. J Anal Toxicol 7:106–110

Wall ME, Brine DR, Jeffcoat AR, Cook CE (1981) Phencyclidine metabolism and disposition in man following a 100 μg intravenous dose. Res Commun Subst Abuse 2:161–172

Wallach JV (2014) Structure activity relationship (SAR) studies of arylcyclohexylamines as N-methyl-D-aspartate receptor antagonists. PhD dissertation, University of the Sciences, Philadelphia

Wallach J, De Paoli G, Adejare A, Brandt SD (2014) Preparation and analytical characterization of 1-(1-phenylcyclohexyl)piperidine (PCP) and 1-(1-phenylcyclohexyl)pyrrolidine (PCPy) analogues. Drug Test Anal 6:633–650

Wallach J, Kang H, Colestock T, Morris H, Bortolotto ZA, Collingridge GL, Lodge D, Halberstadt AL, Brandt SD, Adejare A (2016a) Pharmacological investigations of the dissociative 'legal highs' diphenidine, methoxphenidine and analogues. PLoS One 11:e0157021

Wallach J, Colestock T, Cicali B, Elliott SP, Kavanagh PV, Adejare A, Dempster NM, Brandt SD (2016b) Syntheses and analytical characterizations of N-alkyl-arylcyclohexylamines. Drug Test Anal 8:801–815

Zarantonello P, Bettini E, Paio A, Simoncelli C, Terreni S, Cardullo F (2011) Novel analogues of ketamine and phencyclidine as NMDA receptor antagonists. Bioorg Med Chem Lett 21:2059–2063

Zidkova M, Hlozek T, Balik M, Kopecky O, Tesinsky P, Svanda J, Balikova MA (2017) Two cases of non-fatal intoxication with a novel street hallucinogen: 3-methoxy-phencyclidine. J Anal Toxicol 41:350–354

Zukin SR, Zukin RS (1979) Specific [^{3}H]phencyclidine binding in rat central nervous system. Proc Natl Acad Sci U S A 76:5372–5376

1,2-Diarylethylamine- and Ketamine-Based New Psychoactive Substances

Jason Wallach and Simon D. Brandt

Contents

Abstract

While phencyclidine (PCP) and ketamine remain the most well-studied and widely known dissociative drugs, a number of other agents have appeared since the late 1950s and early 1960s, when the pharmacological potential of this class was first realized. For example, hundreds of compounds have been pursued as part of legitimate research efforts to explore these agents. Some of these found

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their way out of the research labs and onto illicit markets of the 1960s and following decades as PCP analogs. Other "illicit analogs" apparently never appeared in the scientific literature prior to their existence on clandestine markets, thus originating as novel innovations in the minds of clandestine chemists and their colleagues. Like so much else in this world, new technologies changed this dynamic. In the 1990s individuals separated by vast geographical distances could now communicate nearly instantaneously with ease through the Internet. Some individuals used this newly found opportunity to discuss the chemistry and psychoactive effects of dissociative drugs as well as to collaborate on the design and development of novel dissociative compounds. Similar to modern pharmaceutical companies and academic researchers, these seekers tinkered with the structure of their leads pursuing goals such as improved duration of action, analgesic effects, and reduced toxicity. Whether all these goals were achieved for any individual compound remains to be seen, but their creations have been let out of the bag and are now materialized as defined compositions of matter. Moreover, these creations now exist not only in and of themselves but live on further as permutations into various novel analogs and derivatives. In some cases these compounds have made their way to academic labs where potential clinical applications have been identified. These compounds reached wider distribution when other individuals picked up on these discussions and began to market them as "research chemicals" or "legal highs". The result is a continuously evolving game that is being played between legislatures, law enforcement, and research chemical market players. Two structurally distinct classes that have appeared as dissociative-based new psychoactive substances (NPS) are the 1,2-diarylethylamines and β-keto-arylcyclohexylamines. Examples of the former include diphenidine and various analogs such as fluorolintane and *N*-ethyl-lanicemine, and examples of the latter are analogs of ketamine such as methoxetamine, deschloroketamine, and 2-fluoro-2-deschloroketamine. The subject of this chapter is the introduction to some of the dissociative NPS from these classes and their known pharmacology that have emerged on the market in recent years.

Keywords
Clinical · Designer drugs · Diphenidine, ketamine analogs · Dissociatives · Forensic · NMDA receptor · Pharmacology · Toxicology

Acronyms of the Discussed New Psychoactive Substances (NPS)

2-Cl-DPP (2-Cl-DPH)	1-[1-(2-Chlorophenyl)-2-phenylethyl]piperidine
2-F-DPPy	1-[1-(2-Fluorophenyl)-2-phenylethyl]pyrrolidine (fluorolintane)
2-FDCK	2-(2-Fluorophenyl)-2-(methylamino)cyclohexan-1-one
2-MK	2-(2-Methoxyphenyl)-2-(methylamino)cyclohexan-1-one
2-MXP	1-[1-(2-Methoxyphenyl)-2-phenylethyl]piperidine
2-oxo-PCA	2-Amino-2-phenylcyclohexan-1-one

2-oxo-PCE	2-(Ethylamino)-2-phenylcyclohexan-1-one
2-oxo-PCPr	2-Phenyl-2-(propylamino)cyclohexan-1-one
2-TFMDCK	2-(Methylamino)-2-[2-(trifluoromethyl)phenyl] cyclohexan-1-one
3-MeO-PCP	1-[1-(3-Methoxyphenyl)cyclohexyl]piperidine
3-MXP	1-[1-(3-Methoxyphenyl)-2-phenylethyl]piperidine
4-MeO-PV8	1-(4-Methoxyphenyl)-2-(pyrrolidin-1-yl)heptan-1-one
4-MeO-PV9	1-(4-Methoxyphenyl)-2-(pyrrolidin-1-yl)octan-1-one
4-MXP	1-[1-(4-Methoxyphenyl)-2-phenylethyl]piperidine
5F-ADB	Methyl (2*S*)-2-[[1-(5-fluoropentyl)-1*H*-indazole-3-carbonyl]amino]-3,3-dimethylbutanoate
5F-AMB	Methyl (2*S*)-2-[[1-(5-fluoropentyl)-1*H*-indazole-3-carbonyl]amino]-3-methylbutanoate
5/6-APB	1-(1-Benzofuran-5-yl)propan-2-amine or 1-(1-benzofuran-6-yl)propan-2-amine
AB-CHMINACA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMT	1-(1*H*-Indol-3-yl)propan-2-amine (α-methyltryptamine)
Br-MXE	2-(2-Bromo-5-methoxyphenyl)-2-(ethylamino) cyclohexan-1-one
DCK	2-(Methylamino)-2-phenylcyclohexan-1-one
DPE (NEDPA)	*N*-Ethyl-1,2-diphenylethanamine (ephenidine)
DPiP (NPDPA)	*N*-(1,2-Diphenylethyl)propan-2-amine
DPP (1,2-DEP)	1-(1,2-Diphenylethyl)piperidine (diphenidine)
DPPy (1,2-DEPy)	1-(1,2-Diphenylethyl)pyrrolidine
FXE	2-(Ethylamino)-2-(3-fluorophenyl)cyclohexan-1-one (fluoroxetamine)
MK-801	(+)-10,11-Dihydro-5*H*-5,10-epiminodibenzo[*a*,*d*][7] annulene (dizocilpine)
MXE	2-(Ethylamino)-2-(3-methoxyphenyl)cyclohexan-1-one (methoxetamine)
MXM (MMXE)	2-(3-Methoxyphenyl)-2-(methylamino)cyclohexan-1-one (methoxmetamine)
MXP	1-[1-(2-Methoxyphenyl)-2-phenylethyl]piperidine
PCA	1-Phenylcyclohexan-1-amine
PCE	*N*-Ethyl-1-phenylcyclohexan-1-amine
PCP	1-(1-Phenylcyclohexyl)piperidine (phencyclidine)
PV9	1-Phenyl-2-(pyrrolidin-1-yl)octan-1-one

1 Introduction

One of the terms used to describe the effects induced by phencyclidine (PCP, Fig. 1) and ketamine (Fig. 2) is "dissociative anesthetic," a term coined by Toni Domino in an effort to choose a name that describes the unique anesthetic effects of this

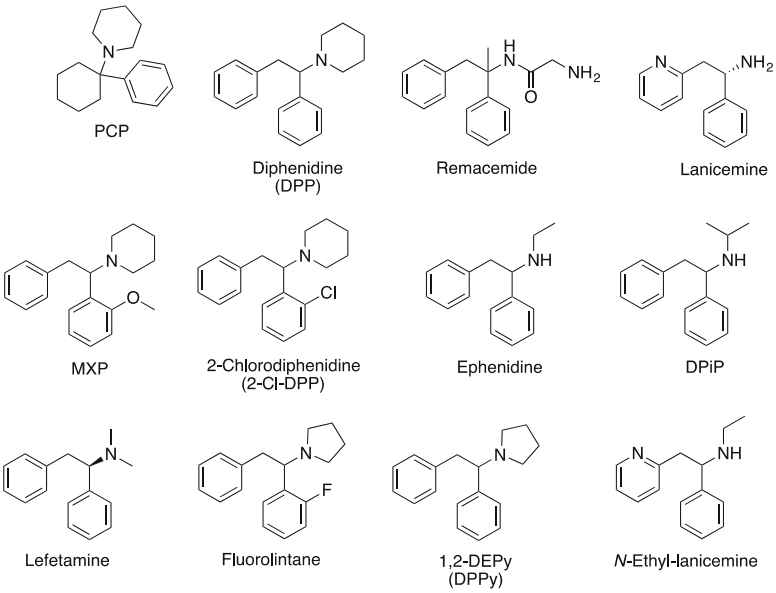

Fig. 1 Phencyclidine (PCP) and a selection of 1,2-diarylethylamines that act as NMDA antagonists. Most of these are available as research chemicals

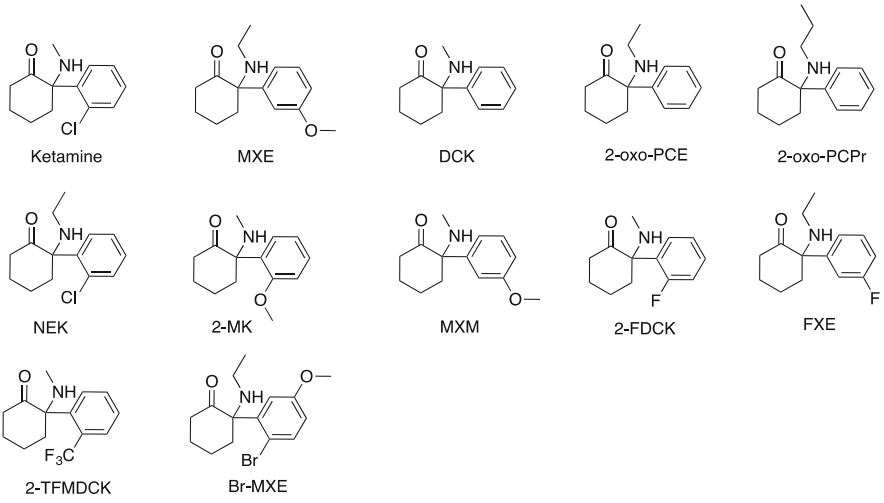

Fig. 2 Ketamine and representative ketamine analogs. Most of these are available as research chemicals

pharmacological class (Domino 2010). The term has since been abbreviated to "dissociative" to account for the wide variety of effects induced by these substances (Morris and Wallach 2014). Effects seen with dissociatives often include stimulation at lower doses with higher doses often inducing sedation, amnesia, and anesthesia.

At lower doses, the intoxication is commonly compared to ethanol and/or nitrous oxide especially. Dose-dependent perceptual alterations in all sensory modalities including visual and auditory allocations may occur. Somatosensory, proprioceptive, and tactile distortions and hallucinations are particularly common. Cognitive effects include depersonalization, derealization, and loss of ego boundaries as well as altered thought patterns, associative thoughts, ideas of reference, unusual thoughts, and, in some cases, delusions and paranoia (Pomarol-Clotet et al. 2006; Morris and Wallach 2014).

Dissociative drugs induce their unique spectrum of subjective effects likely through a shared pharmacological mechanism. While dissociative drugs interact with a number of CNS targets, it is the antagonism of the *N*-methyl-D-aspartate receptor (NMDAR) that is implicated in mediating, at least in part, the subjective and mind-altering effects of many of these compounds (Morris and Wallach 2014; Lodge and Mercier 2015). However, additional mechanisms such as inhibition of monoamine neurotransmitter transporter activity and interactions with opioid and sigma receptors may contribute to the effects of individual compounds. Users will commonly discuss the subtle differences between individual compounds, and more research is needed in this area to identify potentially relevant polypharmacology and clinically useful features.

Technology has reshaped almost every aspect of society. Drug use and recreational drug markets are no different in this respect. The Internet has played a fundamental role in the origin of, dissemination of information about, and distribution of many new dissociative research chemicals (Morris and Wallach 2014). Information exchange about the effects and circumstances of drug use can also provide opportunities for exploring harm reduction advice shared between users, at least between those who engage in online technology as recently discussed within the context of dissociative NPS use (Hearne and Van Hout 2016). Examples also exist where users of dissociative substances make clear references to self-medication and treatment (Morris and Wallach 2014), adding to speculations and discussions around the mechanisms of action associated with some of these substances and how these might affect the users' conditions (Coppola and Mondola 2012, 2013). Whether these speculations and discussions will lead to real-life clinical breakthroughs is unknown, but these reports should perhaps not be discounted. Dissociative agents have shown efficacy and potential in a wide area of therapeutic areas (e.g., Morris and Wallach 2014 and examples cited in this chapter).

Within the narrative of this chapter, dissociative NPS have been separated into two structural classes: 1,2-diarylethylamines (e.g., diphenidine, Fig. 1) and β-keto-arylcyclohexylamines (e.g., MXE, Fig. 2). Arylcyclohexylamines such as 3-MeO-PCP have been covered elsewhere (Wallach and Brandt 2018). The subject of this chapter is the introduction to some of the dissociative NPS from these classes that have emerged on the market in recent years.

2 1,2-Diarylethylamines

1,2-Diarylethylamines contain two aryl groups vicinally connected to an ethylamine side chain (Fig. 1). The 1,2-diarylethylamine template has been investigated in various areas of scientific research for several decades (e.g., Tainter et al. 1943; Dodds et al. 1945; Heinzelman and Aspergren 1953; Morris and Wallach 2014). More recently, several 1,2-diarylethylamines including remacemide and lanicemine (AZD6765) have been investigated in clinical trials where they have shown promise for use in therapeutic indications including depression, seizure, stroke, and neurodegenerative disorders (Palmer and Hutchison 1997; Zarate et al. 2013; Sanacora et al. 2014). A related compound is lefetamine which has been used as an analgesic drug (Wink et al. 2014). The structurally related compound 1-cyclohexyl-4-(1,2-diphenylethyl)piperazine (MT-45) was shown to be a potent synthetic opioid and appeared as a research chemical in its own right (WHO 2015).

The first 1,2-diarylethylamine to be sold specifically as a dissociative research chemical beginning in 2013 appears to have been diphenidine (Fig. 1) (Morris and Wallach 2014). The related compound ephenidine was reported prior to this in a 2008 seizure in Germany (Westphal et al. 2010). However, the use of ephenidine as a dissociative NPS does not appear to have occurred until early 2015. Shortly following the introduction of diphenidine, its 2-methoxy derivative methoxphenidine (MXP, 2-MXP) appeared (Morris and Wallach 2014). From the perspective of the UK market, the choice of offering 1,2-diarylethylamine research chemicals was a response to the introduction of legislation that placed substances derived from 1-phenylcyclohexanamine and 2-amino-2-phenylcyclohexanone (β-keto-arylcyclohexylamine) structural classes under legislative control in 2013 (UK S.I. No. 239 2013). This development was however foreseen by research chemical vendors who waited for legislation to enter into force followed immediately by offering diphenidine for sale. Diphenidine and other 1,2-diarylethylamines were known in the literature as NMDAR antagonists (Gray and Cheng 1989; Berger et al. 2009), and it is likely they were selected based on this property in an attempt to offer non-controlled alternatives to PCP- and ketamine-derived NPS.

2.1 Diphenidine

Diphenidine (DPP) appears to have been first synthesized in 1924 by Christiaen (1924) using a modified Bruylants reaction. Incredibly, this is the same reaction later used by V. Harold Maddox in 1956 that led to the serendipitous discovery of PCP (Morris and Wallach 2014). Other reports describing the preparation and also various analytical characterizations of diphenidine have subsequently been published (e.g., Goodson and Christopher 1950; Stewart and Hauser 1955; Le Gall et al. 2006; Hesp and Stradiotto 2010; Wallach et al. 2015; Geyer et al. 2016; Xie and Dixon 2017). Diphenidine appears to be the first 1,2-diarylethylamine dissociative research chemical to see wider distribution (Morris and Wallach 2014). Its detection was first reported to the European Monitoring Centre for Drugs and Drug Addiction

(EMCDDA) in January 2014 (EMCDDA–Europol 2015). Diphenidine induces dissociative effects at doses starting around 50–100 mg and is active by oral and parenteral routes although nasal insufflation has been described as irritating. Reports of high-dose ingestion do occur, with some describing unpleasant experiences. The duration of action has been given as 3–6 h and 2–5 h based on investigation of user reports online (Morris and Wallach 2014; Beharry and Gibbons 2016). Others have suggested common oral doses of diphenidine and the related MXP to be around 50–150 mg with duration of 3–7 h although, again, higher doses are commonly described (Helander et al. 2015).

2.1.1 Pharmacokinetics

Similar to PCP, diphenidine is a tertiary amine and weak base. Diphenidine is likewise highly lipophilic with an estimated cLogP of 5.05 (Chemdraw Ultra). For comparison, the LogP of PCP is 5.1 (Kamenka and Geneste 1981). Consistent with this, high distribution has been seen into adipose tissue of postmortem samples. For example, diphenidine was detected in blood, urine, and several solid tissues in an autopsy of a fatal case involving diphenidine and synthetic cannabinoid receptor agonists 5-F-AMB and AB-CHMINACA. Eight solid tissues were analyzed in this study including the adipose tissue, brain, heart muscle, liver, kidney, spleen, lung, and pancreas. The highest concentration of diphenidine (11,100 ng/g) was detected in adipose tissue (Hasegawa et al. 2015). Diphenidine has also been detected in hair samples 49 days following single administration (123, 79, and 89 pg/mg in first three proximal segments) (Alvarez et al. 2017), whereas the detection of 4,400 pg/mg was reported in another study (Salomone et al. 2016).

The metabolic transformation of diphenidine has been investigated using CYP450 isozyme preparations, pooled human liver microsomes, and cytosol and following administration to rats (male Wistar) for urinalysis (Wink et al. 2016). Mono- and bis-hydroxyl and oxo-piperidine metabolites were detected using the human liver preparations. CYP1A2, CYP2B6, CYP2C9, and CYP3A4 were found to be capable of catalyzing formation of the hydroxy-aryl, hydroxy-piperidine, and bis-hydroxypiperidine metabolites, whereas CYP2D6 produced hydroxy-aryl and hydroxy-piperidine metabolites. In rat urine, the major phase I metabolites detected were those resulting from mono- and bis-hydroxylation, dehydrogenation, oxo-piperidine, *N*,*N*-bis-dealkylation, and various combinations of these. Phase II metabolites included glucuronide conjugates of the phase I metabolites and/or methylation of one of the bis-hydroxy-aryl groups (Wink et al. 2016).

Diphenidine and the hydroxyl-piperidine diphenidine metabolite were detected at trace concentrations in a fatal intoxication case associated with MXP although it was not possible to discern whether these derived from MXP transformation or whether this was a reflection of diphenidine co-ingestion (Elliott et al. 2015). Two major metabolites detected in blood and urine in a fatal case involving diphenidine were those resulting from mono-hydroxylation of the piperidine ring and mono-hydroxylation of a phenyl ring. Dihydroxy-dehydrogenated as well as dihydroxylated metabolites (positions not determined but dehydrogenation appeared to occur mainly on the piperidine ring) were also detected (Minakata et al. 2015).

Mono- and dihydroxy metabolites of diphenidine, involving the piperidine and both phenyl rings, were detected in blood and urine from a fatal case involving diphenidine and the synthetic cannabinoid receptor agonist 5F-ADB (Kusano et al. 2017).

2.1.2 Pharmacodynamic Effects In Vitro

Diphenidine has been found to have high affinity for the PCP binding site of NMDAR (Wallach et al. 2016; Gray and Cheng 1989; Berger et al. 2009) (Table 1). Diphenidine contains a stereogenic carbon center and is thus chiral containing two enantiomers. In two studies, large differences in NMDAR affinity were reported between diphenidine enantiomers, with (+)-(*S*-)-diphenidine showing substantially higher affinity than the (−)-(*R*-) enantiomer (Table 1) (Gray and Cheng 1989; Berger et al. 2009). Diphenidine acts as an NMDAR antagonist likely through an uncompetitive channel-blocking effect as it was found to block NMDAR-mediated field excitatory postsynaptic potentials (fEPSPs) in rat hippocampal slices (1 and 10 μM) in a manner consistent with a channel blocker. In addition to diphenidine, a number of related compounds and known NMDAR antagonists were recently evaluated for effects on NMDAR-mediated fEPSPs. The rank order of potency for inhibition was found to be MK-801 > PCP > 2-Cl-DPP ≥ DPP ≥ 3-MXP ≥ 2-MXP > ketamine > 4-MXP ≥ memantine, which closely paralleled NMDAR affinities (Wallach et al. 2016).

Diphenidine showed affinities for (Table 1) and exhibited reuptake inhibition properties at human monoamine transporters NET and DAT in transfected HEK293 cells (IC_{50} = 9,250 and 1,990 nM, respectively; SERT IC_{50} > 10,000 nM) (Wallach et al. 2016). Similar results (Table 1) were reported by Luethi et al. (2018) using comparable methods (NET IC_{50} = 3,300 nM, DAT IC_{50} = 3,400 nM, SERT IC_{50} = 675 μM) (Luethi et al. 2018). However, diphenidine did not cause monoamine ([^{3}H]NA, [^{3}H]DA, or [^{3}H]5-HT) efflux in the same transporter-transfected HEK293 cells (Luethi et al. 2018). In addition to activity at NMDAR and monoamine transporters, diphenidine showed low μM affinities at human 5-HT receptor subtypes (5-HT_{1A} and 5-HT_{2A}), alpha-adrenergic receptor subtypes (α_{1A}, α_{2A}, α_{2B}, and α_{2C}), various histamine receptor subtypes, muscarinic subtypes, and the kappa opioid receptor (KOR) with some differences between studies (Wallach et al. 2016; Luethi et al. 2018). Diphenidine also showed affinity for sigma-1 (K_i = 290 nM) and sigma-2 receptors (K_i = 193 nM) (Wallach et al. 2016). Finally, diphenidine lacked notable affinity (>15 μM) at rat and mouse trace amine-associated receptor one (TAAR-1) in transfected HEK293 cells (Simmler et al. 2016). As monoamine reuptake inhibition and the other effects discussed are reduced in potency relative to NMDAR antagonism, their contribution to the drug effects of diphenidine is unclear, although a contribution, particularly in cases of high doses or overdoses, cannot be excluded.

Table 1 Receptor binding affinities of 1,2-diarylethylamines at key CNS receptor sites

Compound	NMDAR	NET	DAT	SERT
Diphenidine	$K_i = 18.2$ nM Wallach et al. (2016) $K_i = 39$ nM Gray and Cheng (1989)	$K_i = 2{,}808$ nM Wallach et al. (2016) $K_i = 3{,}400$ nM Luethi et al. (2018)	$K_i = 317$ nM Wallach et al. (2016) $K_i = 230$ nM Luethi et al. (2018)	$IC_{50} > 10{,}000$ nM Wallach et al. (2016) $K_i = 27$ μM Luethi et al. (2018)
(+)-(*S*)-DPH	$K_i = 130$ nM Berger et al. (2009) $K_i = 25$ nM Gray and Cheng (1989)			
(−)-(*R*)-DPH	$K_i = 5{,}250$, 7,020 nM Berger et al. (2009) $K_i = 2{,}900$ nM Gray and Cheng (1989)			
2-MXP	$K_i = 36$ nM Wallach et al. (2016) $K_i = 170$ nM Gray and Cheng (1989)	$IC_{50} > 10{,}000$ nM Wallach et al. (2016) $K_i = 6{,}900$ nM Luethi et al. (2018)	$K_i = 2{,}915$ nM Wallach et al. (2016) $K_i = 4{,}800$ nM Luethi et al. (2018)	$IC_{50} > 10{,}000$ nM Wallach et al. (2016) $K_i = 20$ μM Luethi et al. (2018)
Ephenidine	$K_i = 66$ nM Kang et al. (2017) $K_i = 257$ nM Thurkauf et al. (1989)	$K_i = 841$ nM Kang et al. (2017)	$K_i = 379$ nM Kang et al. (2017)	$IC_{50} > 10{,}000$ nM Kang et al. (2017)

Radioligands and tissue preparations used for NMDAR binding: Wallach et al. (2016) and Kang et al. (2017) [^{3}H]MK-801 in rat forebrain. Gray and Cheng (1989) [^{3}H]TCP in whole rat brain. Radioligands and tissue preparations used for DAT, NET, and SERT: Luethi et al. (2018), Wallach et al. (2016), and Kang et al. (2017). Radioligands: *N*-methyl-[^{3}H]nisoxetine (NET), [^{3}H] WIN35,428 (DAT), [^{3}H]citalopram (SERT) in HEK293 cells transfected with human transporters *NMDAR* *N*-methyl-D-aspartate receptor, *NET* norepinephrine transporter, *DAT* dopamine transporter, *SERT*, K_i inhibitory constant, IC_{50} half maximal inhibitory constant

2.1.3 Effects In Vivo

Diphenidine significantly disrupted pre-pulse inhibition (PPI) in male Sprague-Dawley rats ($ED_{50} = 9.5$ mg/kg, sc) (Wallach et al. 2016). In comparison, PCP, (*S*)-ketamine, and (*R*)-ketamine were found to have ED_{50} values of 0.88, 2.86, and 6.33 mg/kg, sc for PPI disruption in the same model, respectively (Halberstadt et al. 2016). PPI is a measure of sensorimotor gating, and inhibition of PPI is seen with other NMDAR antagonists in rats and is believed to be predictive of dissociative effects in humans (Halberstadt et al. 2016). The reduced potency of diphenidine

(as well as that of MXP) relative to PCP and ketamine is notable given the high affinity for NMDAR seen (Table 1) and suggests that possible pharmacokinetic effects might influence the potency of 1,2-diarylethylamines in vivo. The reduced potency in PPI is consistent with reports of relatively low potency in humans with common doses of diphenidine around 50–100 mg (see above). The reason for the reduced potency relative to NMDAR affinity warrants further study. Other behavioral effects have been reported with diphenidine. For example, stereotypy was induced in male Sprague-Dawley rats following three different routes of administration: intracerebroventricular (icv, ED_{50} = 220 nmol/rat vs. PCP = 150 nmol/rat), subcutaneous (sc, ED_{50} = 2.9 mg/kg vs. PCP not tested), and intraperitoneal injection (ip, ED_{50} = 2.0 mg/kg vs. PCP not tested). Interestingly, the (+)-(*S*)-enantiomer was more potent in eliciting stereotypic behavior using two of the three routes of injection (icv, ED_{50} = 120 nmol/rat; sc, ED_{50} = 0.78 mg/kg; ip, ED_{50} = 2.1 mg/kg) consistent with a slightly higher NMDAR affinity compared to the racemate (Table 1). In contrast, the ED_{50} for the (+)-(*R*)-enantiomer of diphenidine was given as >40 mg/kg (sc) (Gray and Cheng 1989).

2.1.4 Clinical Toxicology

In the period between January and December 2014, 14 hospitalizations in Sweden were reported to involve diphenidine and 3 involved MXP (see section below). Diphenidine concentrations ranged from 2 to 262 ng/mL (mean 88.4 ng/mL) in serum and between 8 and 19,000 ng/mL (mean 2,213 ng/mL) in urine. In all but two cases, other substances were detected in urine and/or blood. Common clinical features of intoxication with diphenidine and/or MXP included hypertension (76%), tachycardia (47%), anxiety (65%), and altered mental status (65%) including confusion, disorientation, dissociation, and/or hallucinations. Nystagmus (24%), meiosis (35%), and muscle rigidity (24%) were also reported (Helander et al. 2015). A recent case involving a diphenidine intoxication in a 30-year-old male was described in which the patient exhibited "agitation, disorientation and altered consciousness state," tachycardia, increased respiration, miosis, muscle rigidity, and signs of metabolic acidosis and rhabdomyolysis. This patient was hospitalized after being found in a "confused and agitated state" and was unable to communicate. Diphenidine concentrations in plasma and urine were 308 and 631 ng/mL, respectively (methylphenidate and diclazepam were also found in plasma) (Gerace et al. 2017). A fatal intoxication of a 53-year-old male involving the synthetic cannabinoid receptor agonist 5F-ADB and diphenidine was reported in 2017. Postmortem blood concentrations (right heart) were found to be 12 ng/mL for diphenidine and 0.19 ng/mL 5F-ADB (Kusano et al. 2017). In an autopsy case of a 30-year-old male, analyses of various tissue and biofluid samples obtained revealed the detection of AB-CHMINACA, 5F-AMB, and diphenidine. Whereas the first two drugs were not detected in femoral and heart blood and urine (but other tissues, e.g., the brain, adipose tissue, and others), the diphenidine concentration was determined at 715 ng/mL in femoral blood (right heart blood, 707 ng/mL; left heart blood, 923 ng/mL; urine, 376 ng/mL). Moreover, adipose tissue revealed a particularly high diphenidine concentration of 11,100 ng/g, the highest of all tissues analyzed (Hasegawa et al. 2015). The same case samples were analyzed using an alternative analytical

technique, which revealed diphenidine concentrations in blood (right atrium) and in urine of 726 and 304 ng/mL (Minakata et al. 2015). Another fatal case was reported involving diphenidine, three synthetic cathinones, ethanol, and therapeutic concentrations of benzodiazepines in Japan involving a female individual in her 30s. The concentrations of 4-MeO-PV8, PV9, 4-MeO-PV9, and diphenidine detected in femoral blood were 2,690, 743, 261, and 1,380 ng/mL, respectively. The only findings reported included pulmonary congestion and edema (Kudo et al. 2015). A death involving a female in her 20s associated with multiple drug intoxication was reported to involve the detection of 7-aminoflunitrazepam, 7-aminonimetazepam, chlorpheniramine, and diphenidine in femoral blood at concentrations of 86, 27, 66, and 73 ng/mL, respectively. Congestion and edema were reported (Kinoshita et al. 2017).

2.2 MXP

Shortly following diphenidine, MXP (2-MXP, methoxphenidine) was the second 1,2-diarylethylamine dissociative to become widely available as a research chemical and its history has been described previously (Morris and Wallach 2014). Syntheses and analytical characterizations related to MXP and positional isomers have been described (e.g., Taschwer et al. 2014; McLaughlin et al. 2016; Geyer et al. 2016; Xie and Dixon 2017; Boateng et al. 2018). MXP is psychoactive via oral and parenteral routes although nasal insufflation, similar to diphenidine, has been reported to be unpleasant. Based on reviews of online posts, active doses were reported to start around 30–50 mg with doses of greater than 150 mg being described as strong. The duration of action of MXP has been described as between 6–8 h (Beharry and Gibbons 2016) and 3–7 h (and/or diphenidine; Helander et al. 2015). An evaluation of Internet forum posts suggested that a number of users reported MXP as more pleasurable than diphenidine, with one user calling it an "absolute gem of a chem." Many users appeared to prefer the oral route of ingestion, although other routes were also described (im, intranasal, rectal (plugging), sublingual, and smoking). Doses described generally ranged from 30 to 80 mg although reports of high doses in the hundreds of mg were described. Onset was reported to occur in 30–60 min (dependent on a number of factors including dose, tolerance, and route of administration). Some users described long-lasting and cumulative psychoactive effects with repeated dosing speculating this might have been due to a long half-life. Of note is that a number of users reported interest in the therapeutic use of MXP as a "life enhancer" and antidepressant (Van Hout and Hearne 2015). Desirable effects included stimulant and dissociative effects, euphoria, introspection, out-of-body experiences, auditory and visual hallucinations, enhanced tactile sensations, kinesthetic effects, and spatial distortion. Negative side effects reported included "physical sensations of chest pain, palpitations, facial flushing, cold extremities, respiratory difficulties, hyperthermia, and spasms." Some users also reported incidences of urinary retention, hypertension, and seizures. Cognitive impairment, muscle cramps, and "numbness in left-side extremities" were also described by some users to last several days following ingestion (Van Hout and Hearne 2015).

2.2.1 Pharmacokinetics

Systematic studies investigating the metabolism of MXP have not been published so far. Investigations of biofluids derived from fatal intoxication cases associated with MXP uncovered the detection of hydroxy-MXP (piperidine ring) as the main metabolite in blood and urine. *O*-Demethyl-MXP and hydroxyl-*O*-demethyl-2-MXP (piperidine ring) were also detected. Notably, diphenidine and hydroxy-diphenidine (piperidine ring) were reported in what appeared to be trace concentrations in these cases although it was not possible to determine whether these resulted from metabolism of MXP or from ingestion of diphenidine as a separate substance (Elliott et al. 2015). Hydroxy-MXP, dihydroxy-MXP, and hydroxyl-demethyl-MXP metabolites (position of hydroxylation undetermined) were also detected in urine of a 35-year-old man who was hospitalized resulting from MXP intoxication (Lam et al. 2016). Three hydroxylation products, *O*-demethyl-MXP, and three glucuronidated hydroxylation products (positions of modifications not specified) were detected in a urine sample collected from an acute intoxication case (Hofer et al. 2014). In all cases (Hofer et al. 2014; Lam et al. 2016; Elliott et al. 2015), prescription medicines were also detected.

2.2.2 Pharmacodynamic Effects In Vitro

MXP was found to be a high-affinity NMDAR antagonist (Kang et al. 2017; Wallach et al. 2016; Gray and Cheng 1989; Berger et al. 2009) (Table 1). Correspondingly, MXP blocked NMDAR-mediated fEPSPs in rat hippocampal slices (1 and 10 μM) with no effect on AMPA receptor-mediated fEPSPs at 50 and 100 μM (Wallach et al. 2016). The 3- and 4-MeO isomers of MXP have also been investigated. Interestingly, the rank order of potency for NMDAR affinity followed the same pattern seen with arylcyclohexylamines (3-MeO- > 2-MeO- > 4-MeO-) suggesting overlapping structure activity relationships of the benzylpiperidine moieties between arylcyclohexylamines and 1,2-diarylethylamines (Gray and Cheng 1989; Wallach 2014; Wallach et al. 2016). Remarkably, 2-Cl-DPP was found to have the highest NMDAR affinity of the five 1,2-diarylethylamines tested consistent with an earlier study from a patent (Gray and Cheng 1989; Wallach et al. 2016). MXP showed affinities (Table 1) and reuptake inhibition at human monoamine transporters NET (IC_{50} = 35.2 μM, IC_{50} = 7,800 nM) and DAT (IC_{50} = 30 μM, IC_{50} = 65 μM) expressed in HEK293 cells (Wallach et al. 2016; Luethi et al. 2018). Significantly reduced effects were reported at SERT (IC_{50} > 10,000 nM, IC_{50} = 741 μM) (Wallach et al. 2016; Luethi et al. 2018). The low activity at SERT relative to DAT and NET was observed with five 1,2-diarylethylamines evaluated including diphenidine, 2-MXP, 3-MXP, 4-MXP, and 2-Cl-DPP. 3-MXP had the highest affinities (K_i = 88 and 325 nM, respectively) and inhibition potencies (IC_{50} = 587 and 2,710 nM) at DAT and NET, of the series (Wallach et al. 2016). Interestingly, explorations of the *N*-(1,2-diphenylethyl)piperazine template including substituents at both the benzyl ring and phenyl ring gave rise to monoamine reuptake inhibitors with selectivity for serotonin and norepinephrine over dopamine transporters (Fray et al. 2006a, b). As with diphenidine, MXP did not cause monoamine ([^{3}H]NA, [^{3}H]DA, or [^{3}H]5-HT) efflux in monoamine transporter-transfected HEK293 cells

(Luethi et al. 2018). Although some discrepancies exist between the two studies, MXP showed low μM affinities for 5-HT receptor subtypes (5-HT_{2A} and 5-HT_{2c}), α-adrenergic receptor subtypes (α_{2A}), histamine receptor subtypes, muscarinic subtypes, and KOR (Wallach et al. 2016; Luethi et al. 2018). Affinities for sigma-1 (K_i = 124 nM) and sigma-2 (K_i = 508 nM) were also seen for MXP (Wallach et al. 2016), and MXP lacked significant affinity (>15 μM) at rat and mouse trace amine-associated receptor one (TAAR-1) (Simmler et al. 2016).

2.2.3 Effects In Vivo

Similar to diphenidine and other NMDAR antagonists, MXP (20 mg/kg, sc) significantly disrupted PPI in male Sprague-Dawley rats albeit with lower potency than diphenidine (ED_{50} = 9.5 mg/kg, sc). MXP was also less potent in these PPI experiments compared to PCP and ketamine (Halberstadt et al. 2016), which was unexpected based on its high NMDAR affinity, although this was consistent with reduced potency in humans and the same can be said for diphenidine (Wallach et al. 2016).

2.2.4 Clinical Toxicology

What appeared to be the first published fatal cases associated with MXP appeared in 2015, which also included the differentiation between three positional isomers 2-, 3-, and 4-MXP. In the first case, a 34-year-old male, the cause of death was ruled as MXP use and hypertensive heart disease. Mirtazapine, lamotrigine, and citalopram were also detected at therapeutic concentrations. In the second case, a 34-year-old male, diazepam and quinine were also detected. In the absence of other pathological findings, the cause of death was given as "probable methoxyphenidine toxicity." In the third case, a 38-year-old male, risperidone was also detected at a therapeutic concentration, and the cause of death was due to fatal injuries sustained from a fall. MXP concentrations in femoral blood were found to be 24,000, 2,000, and 1,360 ng/mL, respectively (Elliott et al. 2015, 2018).

As described in the diphenidine section, a number of cases involving MXP intoxication were described in Sweden as part of the STRIDA project (Jan–Dec 2014). MXP concentrations were reported to range from 187 to 409 ng/mL in serum and 3 to 8,367 ng/mL (median 610 ng/mL) in urine. The clinical features were consistent with those described above in the diphenidine section (Helander et al. 2015).

A case of MXP intoxication involving a 53-year-old male has been reported. He was found in a "somnolent and confusional state." Reported signs and symptoms included miosis, tachycardia, hypertension, echolalia, confusion, agitation, opisthotonus, nystagmus, and amnesia. This patient was also taking a large number of prescription and non-prescription drugs, which might have contributed to the observed clinical features. MXP was identified qualitatively in plasma and urine (Hofer et al. 2014).

A 35-year-old male, with a history of hypothyroidism, Wolff-Parkinson-White syndrome, adjustment disorder, and alcohol dependence and who had ingested MXP, was found somnolent in the street. He exhibited retrograde amnesia, hypertension (179/95 mmHg), slurred speech, mild hypokalemia, as well as elevated

alanine amino transferase, aspartate aminotransferase, and lactate dehydrogenase levels. In addition, severe rhabdomyolysis (considered multifactorial) and acute kidney injury were noted. MXP (qualitative detection), a methylphenidate metabolite, tramadol, and lorazepam were detected in urine (Lam et al. 2016).

A case report involving the drowning of a 21-year-old male in a bathtub associated with multidrug exposure revealed the detection of lorazepam (5.7 ng/mL), delorazepam (54 ng/mL), amphetamine (64 ng/mL), 4-fluoroamphetamine (2.1 ng/mL), and MXP (190 ng/mL) in femoral blood. Blood alcohol concentration was determined to be 0.93‰ (Grumann et al. 2016).

A 21-year-old male with a history of drug use and bipolar I disorder was hospitalized due to agitation and aggressiveness following regular, self-reported use of MXP. Analytical data recorded from biofluids were not available apart from the confirmation of MXP in the patient's drug sample. Over the course of hospitalization, the authors suggested that the patient might have suffered from withdrawal symptoms (including abdominal pain, vomiting, and low-grade fever (38°C) without infectious diseases) (Champeau et al. 2017).

Unexpected clinical features mimicking an ischemic cerebral disease were observed in a 25-year-old male who presented with the inability to maintain the upright position and exhibited referred hyposthenia at lower limbs after an episode of syncope with secondary head trauma. The patient was described as exhibiting excitatory behavior, psychomotor agitation, confusion, dysarthria, and aphasia. Mild hypertension (150/100 mmHg), heart rate (85 bpm), and a body temperature of 36.5°C were also described. MXP and flubromazepam (NPS benzodiazepine) concentrations in blood were determined at 247 ng/mL and 411 ng/mL, respectively (Valli et al. 2017).

A severe serotonin syndrome was described in a 33-year-old autistic male who ingested one or more tablets containing MXP and tryptamine-based hallucinogen AMT (Chretien et al. 2018). He was found in a state of severe agitation and hospitalized at which point he presented with profuse sudation, hyperthermia, tachycardia (140 bpm), and mydriasis. A Glasgow Coma Score of 10/15 was determined. He also was reported to have hypercapnic acidosis, elevated lactates, renal dysfunction, and rhabdomyolysis. Effects induced by AMT (including inhibition of monoamine oxidase) increase the risk of serotonergic toxicity (Elliott et al. 2013). This patient's "usual treatment" included methadone, loxapine, and lorazepam, which were also detected as were several other substances including mirtazapine, amantadine, and nortriptyline (Chretien et al. 2018)

A 32-year-old male with a history of psychosis and multi-substance use disorder including self-reported use of various dissociative drugs including MXP was hospitalized for treatment of psychotic disorder although it was not specified whether MXP use was detected in biofluids (Caloro et al. 2018).

2.3 Ephenidine

Ephenidine (NEDPA or DPE) was detected in a confiscated sample in Germany in 2008 (Westphal et al. 2010). As stated previously, the availability of ephenidine as a dissociative research chemical, at least based on sale from online retailers or discussion of use on drug forums, did not appear to occur until around 2014 (Beharry and Gibbons 2016). The synthesis and analytical characterization of ephenidine has been reported (Goodson et al. 1946; Campbell et al. 1948; Goodson and Christopher 1950; Novelli and Huidobro 1963; Marcsekova et al. 2005; Garcia Ruano et al. 2009; Kang et al. 2017; Xie and Dixon 2017). Ephenidine has been reported to be an active dissociative through oral and parenteral routes with doses in the 10–100s of mg range, with a threshold dose given as 60 mg. Doses over 200 mg were considered "heavy" according to users on discussion forms. The onset has been said to be slow by some users especially with oral ingestion, and the duration of effects have been reported to be around 5–7 h although higher doses may result in longer duration. Users seem to like the effects and in some cases more so than some other dissociatives (Beharry and Gibbons 2016). The related *N*-(1,2-diphenylethyl) propan-2-amine (NPDPA or DPiP) was also detected along with ephenidine in confiscated samples in Germany in 2008 (Westphal et al. 2010). Currently, DPiP does not appear to be available as a research chemical.

2.3.1 Pharmacokinetics

The metabolism of ephenidine and DPiP was investigated in male Wistar rats using urinalysis. The phase I metabolic transformations detected in urine included *N*-dealkylation (leading to the same primary amine metabolite, 1,2-diphenethylamine (DPA)), mono- and bis-aryl-hydroxylation on the benzyl ring, and combinations of these. Phase II metabolites included glucuronidation, methylation, and sulfation of phase I metabolites (Wink et al. 2014). Further work carried out by Wink et al. established the contributions from ten human CYP450 isozymes to the *N*-dealkylation of ephenidine, DPiP, and lefetamine. For ephenidine net clearances were calculated using a relative activity factor approach as follows: 27% (CYP1A2), 30% (CYP2B6), 23% (CYP2C19), 4% (CYP2D6), and 17% (CYP3A4) (Wink et al. 2015). The net clearances calculated for the *N*-dealkylation of DPiP were found to be 18% (CYP1A2), 24% (CYP2B6), 28% (CYP2C19), and 30% (CYP3A4). For lefetamine, the values were 8% (CYP1A2), 72% (CYP2B6), 2% (CYP2C19), 1% (CYP2D6), and 17% (CYP3A4) (Wink et al. 2015). Ephenidine and DPiP have also been evaluated for substrate activity at the human efflux transporter P-glycoprotein (Pgp). A K_m value of 4.6 μM was determined for DPiP, whereas ephenidine lacked substantial substrate activity at concentrations as high as 500 μM (Meyer et al. 2013b).

2.3.2 Pharmacodynamic Effects In Vitro

Ephenidine has high-to-modest affinity at the PCP binding site of NMDAR (Table 1) in rat brain labeled by [^{3}H]MK-801 (Thurkauf et al. 1989 (details not reported); Kang et al. 2017). NMDAR antagonism was observed through blockade of

NMDAR-mediated fEPSPs in rat hippocampal slices at 1, 10, and 30 μM. No effect on AMPA receptor-mediated fEPSPs at 50 μM was demonstrated consistent with MXP (Kang et al. 2017, Wallach et al. 2016). Voltage-dependent NMDAR blockade for ephenidine was found to be similar to ketamine, memantine, MK-801, and Mg^{2+} where outward currents were less affected than inward. Specifically, ephenidine reduced inward current at negative holding potentials consistent with a channel blocker effect. Ephenidine had a rectification index, between that of Mg^{2+} and ketamine (Kang et al. 2017). Importantly, the voltage dependency of NMDAR blockage has been related to therapeutic potential (Frankiewicz et al. 1996). Ephenidine also had a slower onset of NMDAR fEPSP blockade compared to ketamine at equivalent concentrations in this study (Kang et al. 2017). This slower onset was seen with other 1,2-diarylethylamines tested as well as MK-801 and PCP and is suggestive of altered blocking kinetics relative to ketamine (Wallach et al. 2016). Consistent with other NMDAR antagonists (Frankiewicz et al. 1996), ephenidine blocked long-term potentiation induction induced by theta burst stimulation at 10 μM in CA1 region of rat hippocampal slices (Kang et al. 2017). Similar to several related 1,2-diarylethylamines, ephenidine was observed to have modest affinities at human monoamine transporters DAT and NET but not SERT expressed in HEK293 cells (Table 1) (Kang et al. 2017). Modest affinity was also seen for sigma-1 and sigma-2 receptors (K_i = 629 and 722 nM, respectively) (Kang et al. 2017). The *N*-dealkylation metabolite, DPA, has been reported to have modest affinity for NMDAR (K_i = 690 nM) (Thurkauf et al. 1989) and therefore could contribute to the pharmacological effects of ephenidine.

2.3.3 Effects In Vivo

Along with a number of related 1,2-diarylethylamines, ephenidine was evaluated for its sympathomimetic and bronchodilating properties. In dogs (5–15 mg/kg, iv), ephenidine produced "a fall in blood pressure, which was sometimes followed by a rise of 20–30 mmHg" (details not reported). Low-potency stimulant properties were observed as defined by an increase in spontaneous activity in "white rats" following subcutaneous administration. The threshold dose for ephenidine was 8 mg/kg (compared to 0.25 mg/kg for (*S*)-amphetamine), whereas the maximum peak effect was observed at 128 mg/kg compared to a dose of 2 mg/kg of (*S*)-amphetamine that also displayed twice the response at the maximum dose in terms of spontaneous activity (Tainter et al. 1943). Ephenidine was also part of a series of 1,2-diarylethylamines evaluated in the mid-1940s for analgesic activity in mice although details on the results were not included (Goodson et al. 1946). In an in vivo model used to evaluate potential dopaminergic anti-Parkinson effects (Sprague-Dawley rats lesioned unilaterally with 6-hydroxydopamine in the nigrostriatal system), ephenidine was inactive at a dose of 320 μmol/kg (ip), whereas some aminotetralin derivatives did show dopaminergic effects under these conditions (Cheng et al. 1976). An LD_{50} value in white rat (iv) was determined for ephenidine to be 55 mg/kg (Tainter et al. 1943). In a clinical study in cancer patients with intractable pain, DPA was given orally (200 mg, 3 h intervals) and found to display analgesic properties. However, mental confusion developed after about 1 h.

In healthy subjects, similar doses were reported to produce elation and slight muscular incoordination comparable to effects induced by ethanol. Most patients refused receiving DPA again after receiving a series of doses. Interestingly, the β-hydroxy derivative of DPA (2-amino-1,2-diphenylethanol) (200–400 mg, 4-hourly, po) provided complete pain relief in 14 patients without undesirable side effects (Dodds et al. 1944). As part of a study to evaluate anorectic properties, DPA, among other ring-substituted 1,2-diphenylethan-1-amine analogs, reduced food intake in female Sprague-Dawley rats (ip) without significantly affecting motor activity (Ghosh et al. 1978).

2.3.4 Clinical Toxicology

No reports associated with ephenidine intoxication or fatal overdoses could be identified.

2.4 Other 1,2-Diarylethylamines

2.4.1 Fluorolintane

Fluorolintane appears to have first been marketed as a research chemical around 2015, and limited information available from online posts indicate that fluorolintane might show dissociative effects, for example, at doses from 100–400 mg with a 2–4 h duration. Stimulating effects at 100 mg were also mentioned (Anonymous 2015b). Some analytical data are available in the public domain (National Slovenian Forensic Laboratory 2016), and the synthesis of fluorolintane has been recently described (Xie and Dixon 2017). While fluorolintane appears to have emerged as a novel research chemical recently with no pharmacological data reported in the scientific literature, the defluoro analog of fluorolintane (DPPy, Wallach et al. (2015)), which is also the pyrrolidine analog of diphenidine (DPP), has been prepared and investigated for a variety of potential clinical applications (e.g., Heinzelman and Aspergren 1953; Aspergren and Heinzelman 1963, 1964; Kasé et al. 1963; Yuizono et al. 1970). The reported affinity of DPPy for NMDAR was surprisingly low (K_i = 16,000 nM) (Gray and Cheng 1989), which suggests that further studies are warranted to confirm this finding.

2.4.2 *N*-Ethyl-Lanicemine

One of the more recent 1,2-diarylethylamines sold as a research chemical is *N*-ethyl-lanicemine (Fig. 1). This compound is the *N*-ethyl derivative of lanicemine (AZD6765), a low-trapping NMDAR antagonist (K_i = 0.56–2.1 μM) developed by AstraZeneca and which has been investigated clinically for depression (Zarate et al. 2013; Sanacora et al. 2014; Machado-Vieira et al. 2017). At doses up to 150 mg, lanicemine showed antidepressant effects in treatment-resistant major depressive disorder in randomized trials with minimal dissociative effects (Zarate et al. 2013; Sanacora et al. 2014). By contrast, *N*-ethyl-lanicemine was shown to be a high-trapping NMDAR antagonist, using whole cell recordings in cultured rat cortical neurons, which was suggested to "produce significant amounts of

PCP-like behaviors in rats at lower doses" (Mealing et al. 2001). It is likely that *N*-ethyl-lanicemine might have dose-dependent dissociative effects in humans, but further research is needed to confirm this. A sample of material sold as *N*-ethyl-lanicemine was tentatively identified using mass spectrometry (HR-MS, GC-MS) and NMR (Wallach, Dybek and Brandt unpublished).

3 β-Keto-Arylcyclohexylamines

β-Keto-arylcyclohexylamines contain a ketone function in the respective 2-position (or β-) of the cyclohexyl ring of arylcyclohexylamines. The contribution of the β-keto group to the structure-activity relationships of these compounds relative to their arylcyclohexylamine counterparts remains largely unpublished, and it is hoped that this situation will change with increased interest in this area.

3.1 History

A patent application initiated by the Parke-Davis pharmaceutical company in 1957 described the synthesis of 2-oxo-PCA as an intermediate to prepare the arylcyclohexylamine PCA (Anonymous 1960). In a 1962 communication to the editors of the *Journal of the American Chemistry Society* (received April 25, 1962), Calvin L. Stevens described a novel application of a rearrangement reaction involving α-amino ketones (Stevens et al. 1962). Though this communication did not include the synthesis of any β-keto-arylcyclohexylamines, it laid the synthetic foundation that would later give rise to systematic investigations involving these compounds. What appears to be the first publication to describe a number of β-keto-arylcyclohexylamines and to recognize their clinical potential was a US patent 3,254,124 filed on June 29, 1962 (assigned to Parke-Davis) (Stevens 1962). This patent (granted on May 31, 1966) described the synthesis of a number of important β-keto-arylcyclohexylamines including ketamine, MXM (methoxmetamine), 2-methoxy-2-deschloroketamine (2-MK), DCK, and 2-oxo-PCE. Many of these compounds were also described in more detail in a number of subsequent publications by the Stevens Group focusing on aminoketone rearrangement reactions from the early to mid-1960s (Stevens et al. 1963, 1965a, b, 1966a, b, c; Stevens 1968). Stevens served as a consultant to Parke-Davis during this time and provided these compounds for pharmacological testing.

3.2 Ketamine

In April, 1962, 2-(2-chlorophenyl)-2-(methylamino)cyclohexanone (ketamine) was submitted by Stevens for testing under the test code name CI-369 (McCarthy 1981). During the early 1960s, Parke-Davis and their collaborators conducted a number of pharmacological investigations into ketamine, which would soon be designated

using the clinical code of CI-581 (Chen 1969; McCarthy et al. 1965; Morris and Wallach 2014). The first human subject received ketamine on August 3, 1964 (McCarthy 1981; Domino 2010).

The data from the first human subjects were soon published (Domino et al. 1965). Additional human studies quickly followed, confirming animal studies and establishing CI-581 to be an effective and safe anesthetic and analgesic with an improved tolerability profile over PCP (Corssen and Domino 1966; Chodoff and Stella 1966; McCarthy et al. 1965). This research led to the marketing of ketamine as Ketalar® in 1969 and and its official FDA approval in the USA for use as a general anesthetic in 1970 (Morris and Wallach 2014). Since this time, ketamine has found widespread use internationally as a general anesthetic and is on the World Health Organization's list of essential medicines. In addition to its anesthetic action, ketamine has potent analgesic actions and is successfully used for this purpose (Clements and Nimmo 1981; Kohrs and Durieux 1998). Ketamine also plays an important role in veterinary medicine (Morris and Wallach 2014).

Apart from uses related to traditional medical procedures and recreational or spiritual drug use (Lilly 1996; Jansen 2004), ketamine has also been evaluated for the treatment of drug dependence and neurotic disorders (Krupitsky et al. 2001, 2007). Most recently, ketamine has been increasingly studied for its potential role as an agent in treatment-resistant depression based on initially promising outcomes (e.g., Katalinic et al. 2013; Vutskits 2018; Zanos et al. 2018). In addition to therapeutic uses, ketamine has shown potential in other clinical research applications. For example, administrations of subanesthetic doses of ketamine have been described to mimic certain aspects of psychosis and schizophrenia including positive psychosis symptoms such as hallucinations and altered thoughts, as well as negative symptoms including impairment of cognition, derealization, depersonalization, and withdrawal (e.g., Krystal et al. 1994; Lahti et al. 1995; Malhotra et al. 1996; Vollenweider et al. 1997; Chambers et al. 1999; Newcomer et al. 1999; Pomarol-Clotet et al. 2006; Gouzoulis-Mayfrank et al. 2005; Lodge and Mercier 2015) though important differences exist between drug-induced effects and this spectrum of disorders (Frohlich and Van Horn 2014). For more information, the history of ketamine including its medical and nonmedical use has been described previously (Morris and Wallach 2014; Domino 2010; Lodge and Mercier 2015).

3.2.1 Pharmacokinetics

Ketamine is a weak base with a pK_a of 7.5 (Dayton et al. 1983). The bioavailability of ketamine through different routes of administration has been investigated and was reviewed by Fourcade and Lapidus (2016). Intramuscular injection results in about 93% of bioavailability relative to iv administration. Oral bioavailability of ketamine was reported around 20% due to a substantial first-pass effect, whereas intranasal and sublingual administration have slightly higher bioavailabilities (50% and 30%, respectively) (Fourcade and Lapidus 2016). Ketamine exhibited low but significant affinity for the efflux transporter Pgp as well as a number of other clinically relevant transport proteins including organic cation transporters (Keiser et al. 2018; Amphoux et al. 2006; Massmann et al. 2014).

Modest plasma protein binding has been seen with ketamine including binding to human plasma proteins (up to 47% bound), however differences between studies exist (Dayton et al. 1983). Ketamine is modestly lipophilic (LogP = 2.18) (Hansch et al. 1987) and has been shown to accumulate in rat brain tissue at concentrations 2.3 times higher than the incubation medium in vitro (Cohen and Trevor 1974). The elimination kinetics of ketamine are biphasic. For example, after intravenous application in human subjects, the initial serum half-life (α-elimination phase) was found to be around 11 min resulting from rapid distribution of the drug from plasma to tissue. The β-elimination phase resulting largely from metabolic clearance has been given as 150 min (iv) (Wieber et al. 1975), 186 min (iv) (Clements and Nimmo 1981), and 155 min (im) (Grant et al. 1981).

The metabolism of ketamine has been explored extensively (Mion and Villevieille 2013; Yanagihara et al. 2001; Hijazi and Boulieu 2002; Li et al. 2013b; Clements and Nimmo 1981). Briefly, the major phase I metabolite in humans and rats is norketamine resulting from *N*-demethylation (Mion and Villevieille 2013; Yanagihara et al. 2001; Hijazi and Boulieu 2002; Li et al. 2013b; Clements and Nimmo 1981; Cohen and Trevor 1974). CYP3A4, CYP2C9, and CYP2B6 have been implicated as key enzymes in transformation of ketamine to norketamine (Yanagihara et al. 2001; Hijazi and Boulieu 2002; Li et al. 2013b). The potential for genetic differences in metabolism of ketamine has been considered with a CYP2B6*6 genotype using human liver microsomes, as well as recombinant enzyme systems and human chronic pain patients (Li et al. 2013b, 2015) although a more recent in vivo study in humans failed to confirm this (Rao et al. 2016). Additional phase I metabolites include those from hydroxylation of the cyclohexanone ring of ketamine and norketamine (NK) to form hydroxylated ketamine and norketamine (HNK) metabolites, respectively, that give rise to pairs of diastereomers (Adams et al. 1981; Rao et al. 2016). The formation of 5,6-dehydro-NK (DHNK) has also been reported (Rao et al. 2016). In one study, DHNK formation has been attributed to nonenzymatic (artificial) degradation of the corresponding HNK species due to analysis by gas chromatography mass spectrometry (GC-MS) (Adams et al. 1981). In a study evaluating a 40 min ketamine infusion (0.5 mg/kg, iv), higher plasma concentrations of DHNK, (2*S*,6*S*;2*R*,6*R*)-HNK, (2*S*,6*R*;2*R*,6*S*)-HNK, and (2*S*,5*S*;2*R*,5*R*)-HNK were detected patients with bipolar depression (BD) compared to patients with major depressive disorder (MDD) who had higher concentrations of (2*S*,6*S*;2*R*,6*R*)-HNK. Notably, MDD patients were required to be medication free for at least 2 weeks (5 weeks for fluoxetine) prior to ketamine infusion, whereas BD patients took either lithium or valproate within a specified range (Zarate et al. 2012). Phase II metabolism of ketamine in humans has been observed, most notably in the form of HNK-glucuronides (Turfus et al. 2009; Moaddel et al. 2010). It should also be noted that differences in metabolism of (*R*)- and (*S*)-ketamine have been observed (Kharasch and Labroo 1992). Finally, tolerance to repeated ketamine doses in rats has been reported to involve increased hepatic metabolism through induction (Livingston and Waterman 1978; Marietta et al. 1976). Efforts have been taken to create analogs of ketamine with altered pharmacokinetics. For example, the evaluation of five aliphatic norketamine esters in rabbits (venous cannulation of marginal

ear vein) revealed that this structural modification gave rise to rapid metabolism via hydrolysis to the corresponding carboxylic acid derivatives, which were inactive in vivo in the models tested (Harvey et al. 2015).

3.2.2 Pharmacodynamic Effects In Vitro

Detailed reviews on the pharmacodynamics of ketamine are available (e.g., Mion and Villevieille 2013; Lodge and Mercier 2015; Fourcade and Lapidus 2016; Laher et al. 2015). Ketamine has moderate affinity for the PCP binding site of NMDAR (Table 2) (Roth et al. 2013; Colestock et al. 2018; more in vitro data reviewed in Hondebrink et al. 2018). Ketamine has been shown to block NMDARs in numerous in vitro and ex vivo models (Anis et al. 1983; Hirota and Lambert 1996; Kang et al. 2017). This blockade has been shown to be use- (uncompetitive) and voltage-dependent (MacDonald et al. 1987; Davies et al. 1988; Kang et al. 2017). Differences in NMDAR affinity and NMDAR channel blocker potency are seen between enantiomers with (*S*)-ketamine being about 2–5× more potent than (*R*)-ketamine (Hirota and Lambert 1996; Zeilhofer et al. 1992; Ebert et al. 1997; Oye et al. 1991, 1992). The major metabolite, norketamine, is also a modest affinity NMDAR antagonist ($K_i = 3{,}600$ nM, [^{3}H]MK-801 in rat brain) although with lesser potency than ketamine (Ebert et al. 1997). The metabolite (2*R*,6*R*)-HNK did not inhibit currents evoked by NMDA application to rat stratum radiatum interneurons in hippocampal slices at >10 μM but has been shown to have activity as a modulator of AMPA (increased frequency and amplitude of AMPA receptor-mediated excitatory postsynaptic potentials from rat CA1 stratum radiatum interneurons), at 10 μM concentration (Zanos et al. 2016). Recently, 50 μM concentrations of HNK were found to block NMDAR currents in cultured hippocampal neurons (C57BL/6 mice, postnatal day 1–3) (Suzuki et al. 2017). Ketamine has been shown to impair LTP induction in the brains of rodents, likely through NMDAR antagonism, using several different experimental designs (e.g., Zhang and Levy 1992; Ribeiro et al. 2014).

Recently, Can et al. (2016) found no significant inhibition of reuptake at human SERT, DAT, or NET by ketamine or several metabolites tested ($IC_{50} > 10{,}000$ nM) in transfected HEK293 cells. Similar results were observed when looking at receptor binding to these human monoamine transporters in transfected HEK293 cells (Roth et al. 2013, 2018). However, at higher concentrations ketamine has been reported to bind to monoamine transporters (expressed in HEK293 cells) though with fairly weak potency with K_i values of 66.8 μM (human NET), 62.9 μM (rat DAT), and 161.7 μM (rat SERT) (Nishimura et al. 1998). A follow-up study found stereospecific effects at DAT with (*S*)-ketamine showing ~eightfold greater affinity than (*R*)-ketamine (Nishimura and Sato 1999). Ketamine was also found to inhibit [^{3}H]5-HT, [^{3}H]DA, and [^{3}H]NA uptake in rat synaptosomal fractions (cerebral cortex) with highest effects observed for serotonin transport (Azzaro and Smith 1977), which was consistent with work published later by Smith et al. (1981) using synaptosomal preparations (Sprague-Dawley, cerebral cortex). Stereospecific effects of ketamine have been seen on both stimulated efflux and reuptake for DA, NE, and 5-HT in rat brain slices (Tso et al. 2004).

Table 2 Receptor binding affinities of β-keto-arylcyclohexylamines at key CNS receptor sites

Compound	NMDAR	NET	DAT	SERT
Ketamine	$K_i = 323.9$ nM Colestock et al. (2018) $K_i = 659$ nM Roth et al. (2013) $IC_{50} = 800$ nM Quirion et al. (1981) $K_i = 607$ nM Tam and Zhang (1988) $K_i = 530$ nM Ebert et al. (1997)	$IC_{50} > 10{,}000$ nM Roth et al. (2013) $K_i = 66.8$ μM Nishimura et al. (1998)	$IC_{50} > 10{,}000$ nM Roth et al. (2018) $K_i = 62.9$ μM Nishimura et al. (1998)	$IC_{50} > 10{,}000$ nM Roth et al. (2013) $K_i = 161.17$ μM Nishimura et al. (1998)
(*S*)-(+)-Ketamine	$K_i = 900$ nM Oye et al. (1991) $K_i = 1{,}200$ nM Oye et al. (1992) $K_i = 300$ nM Ebert et al. (1997)	$K_i = 64.8$ μM Nishimura and Sato (1999)	$K_i = 46.9$ μM Nishimura and Sato (1999)	$K_i = 156$ μM Nishimura and Sato (1999)
(*R*)-(−)-Ketamine	$K_i = 2{,}500$ nM Oye et al. (1991) $K_i = 5{,}000$ nM Oye et al. (1992) $K_i = 1{,}400$ nM Ebert et al. (1997)	$K_i = 68.9$ μM Nishimura and Sato (1999)	$K_i = 390$ μM Nishimura and Sato (1999)	$K_i = 148$ μM Nishimura and Sato (1999)
MXE	$K_i = 259$ nM Roth et al. (2013)	$IC_{50} => 10{,}000$ nM Roth et al. (2013)	$IC_{50} > 10{,}000$ nM Roth et al. (2018)	$K_i = 481$ nM Roth et al. (2013)

Radioligands and tissue preparations used for NMDAR binding: [^{3}H]MK-801 in rat brain (Roth et al. 2013). Oye et al. (1991): [^{3}H]TCP (guinea pig brain). Oye et al. (1992): [^{3}H]MK-801 (guinea pig brain); Tam and Zhang (1988): [^{3}H]MK-801 (human brain); Ebert et al. (1997): [^{3}H]MK-801 (in rat cortex, hippocampus, and striatum). Radioligands and tissue preparations used for DAT, NET, and SERT: Roth et al. (2013, 2018): *N*-methyl-[^{3}H]nisoxetine (NET), [^{3}H]WIN35,428 (DAT), [^{3}H]citalopram (SERT) in HEK293 cells transfected with human transporters. Nishimura et al. (1998) and Nishimura and Sato (1999): ([^{3}H]5-HT, [^{3}H] NE, [^{3}H]DA) in HEK293 cells transfected with human NET, rat DAT, and rat SERT. A summary of in vitro data related to ketamine has been provided by Hondebrink et al. (2018)

It should be noted it is unlikely that monoamine reuptake inhibition contributes much to the pharmacology of ketamine in vivo even at high doses due to the high concentrations needed for such effects. Ketamine can however indirectly alter monoamine turnover (Kokkinou et al. 2018; Laher et al. 2015), and this may occur at physiologically relevant concentrations.

A receptor binding study uncovered that ketamine lacked notable affinity ($IC_{50} > 10{,}000$ nM) for a number of important CNS receptors including receptors for 5-HT, dopamine, norepinephrine, histamine, as well as opioid (MOR, KOR, and DOR) and sigma-1 and sigma-2 receptors (Roth et al. 2013, 2018). At concentrations greater than 10,000 nM, ketamine has been found to interact with a number of receptors. For example, both (*R*)- and (*S*)-ketamine were found to have low affinity toward the μ-opioid receptor (MOR) (28 μM and 11 μM, respectively) and low affinities at DOR and KOR receptors using guinea pig brain homogenate (Hustveit et al. 1995), and this was consistent with results reported elsewhere (Hirota et al. 1999). Likewise, low affinities (>10,000 nM) have been reported at sigma and muscarinic receptors with both enantiomers (guinea pig brain homogenate) (Hustveit et al. 1995; Hirota and Lambert 1996). A 500 nM affinity (K_i) and agonist activity was reported for D_2 receptors in rat brain (Kapur and Seeman 2002). Interestingly, ketamine and PCP were reported in a later study by this group to have even higher affinity (55 nM and 2.7 nM, respectively) and functional activity at the high-affinity state of the D_2 receptor (Seeman et al. 2005). These results however have been considered controversial (Svenningsson et al. 2004), and other studies were inconsistent with these findings (Aalto et al. 2002; Jordan et al. 2006; Can et al. 2016; Roth et al. 2018). Low affinity ($K_i = 15$ μM) and agonist activity was also reported at 5-HT_2 receptors in rat brain (Kapur and Seeman 2002).

The understanding of ketamine pharmacology continues to evolve. One interesting avenue to pursue involves the interaction of ketamine with various ion channels. Although a detailed overview is outside the scope of this chapter, the interaction of ketamine with various ion channels has been reviewed. In summary, ketamine has been found to inhibit a range of channels including those of cations K^+, Na^+, and Ca^{2+} as well as hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (reviewed by Laher et al. 2015). An EC_{50} of 8–16 μM for HCN1-HCN2 channel inhibition has been seen with ketamine using electrophysiological recordings in HEK293 cells transfected with mouse HCN channel constructs. In addition, (*S*)-ketamine was two times more potent than the racemate at inhibiting HCN1-HCN2 channels. Fascinatingly, HCN1 antagonism has been implicated in some of the hypnotic effects of ketamine (see Sect. 3.2.3 below) (Chen et al. 2009).

3.2.3 Effects In Vivo

The effects of ketamine in vivo have been extensively described elsewhere (e.g., Kreuscher 1969; Domino 2018). As an illustration of its potential for positive reinforcement, ketamine has been shown to be self-administered in rhesus monkeys (Moreton et al. 1977) and rats (De Luca and Badiani 2011). Similar to other dissociatives, ketamine dose-dependently disrupts PPI in rats (male Wistar and Sprague-Dawley) (de Bruin et al. 1999; Cilia et al. 2007; Halberstadt et al. 2016).

The effect is stereospecific with (*S*)-ketamine having an ED_{50} of 2.86 mg/kg and (*R*)-ketamine an ED_{50} of 6.33 mg/kg for PPI disruption (Halberstadt et al. 2016). Interestingly, ketamine increased PPI in healthy human male volunteers although this might have been related to the relatively low doses used and higher-dose studies in humans are warranted to explore this further (Abel et al. 2003; Heekeren et al. 2007). Ketamine increases dopamine levels in certain areas of the animal brain in vivo, which might be relevant when considering abuse liability (Kokkinou et al. 2018). In addition the HCN1 receptor has been implicated in the hypnotic effects of ketamine as HCN1 knockout mice show reduced sensitivity to the hypnotic effects of ketamine (Chen et al. 2009).

Ketamine has shown activity in a number of experimental models known to be predictive of antidepressant activities in humans including the forced swim test and tail suspension tests in rodents, with the (*R*)-isomer typically showing more sustained effects (Garcia et al. 2008; Koike and Chaki 2014; Zhang et al. 2014; Salat et al. 2015; Niciu et al. 2014). Ketamine and (*S*)-ketamine have also been shown to have potent analgesic effects in several experimental models in animals including rats but also humans (Clements and Nimmo 1981; Koinig et al. 2000; Holtman et al. 2008).

Ketamine has shown anticonvulsant effects as defined as prevention of the tonic hindlimb extensor reflex in the maximal electroshock seizure test (MES) in mice (ip, $ED_{50} = 43.7$ mg/kg, Parsons et al. (1995); sc, $ED_{50} = 15.1$ mg/kg, Leander et al. (1988)). Ketamine also caused impairment in the rotarod (ip, $ED_{50} = 67.4$ mg/kg), traction reflex ($ED_{50} = 53.8$ mg/kg) (Parsons et al. 1995), and horizontal screen test (sc, $ED_{50} = 23$ mg/kg) (Leander et al. 1988). Potency of ketamine in these models is significantly reduced relative to PCP (Wallach and Brandt 2018). Both MES test and rotarod potency have been found to correlate with NMDAR PCP site affinities (Wallach 2014).

Tolerance to the behavioral effects has been observed with repeated ketamine administration in animals including humans (Cumming 1976; Marietta et al. 1976; Livingston and Waterman 1978; MacLennan 1982). Interestingly, a pharmacokinetic component to tolerance, through self-induction of hepatic metabolism, may be at least partially involved as repeated ketamine treatment led to increased metabolic rates in rats (Marietta et al. 1976; Lin et al. 2015).

3.2.4 Clinical Toxicology

Common symptoms of ketamine intoxication include hallucinations, altered mental status, tachycardia, hypertension, mydriasis, and nystagmus (Weiner et al. 2000; Ng et al. 2010). A controlled study of subanesthetic doses of ketamine revealed dose-dependent increases in blood pressure (Krystal et al. 1994). Elevation in plasma cortisol and prolactin were also reported (Krystal et al. 1994). Though ketamine has a wide safety margin, reports of fatal overdoses have been described. These generally involved behavioral effects and included motor vehicle accidents, drowning, and other accidents (Morgan and Curran 2012). However, fatalities from polydrug use as well as cases involving only ketamine including a ketamine-related homicide have been described (Licata et al. 1994; Moore et al. 1997; Lalonde and Wallage

2004; Morgan and Curran 2012). Postmortem toxicology including blood levels (1.8–27.4 mg/L and 0.6-1.8 mg/L in femoral blood) in six reported fatalities has been reviewed by Watterson (2015).

Ketamine dependence and discontinuation symptoms have been described in a number of cases (e.g., Lim 2003; Critchlow 2006; Pal et al. 2002; Hurt and Ritchie 1994). Discontinuation effects described in a 25-year-old male user of ketamine (6-year history) included anxiety, shaking, sweating, palpitations, low mood, drowsiness, and loss of appetite. The time course was described and it was reported that ethanol and diazepam consumption decreased some of the symptoms experienced (Critchlow 2006).

Ketamine has shown toxicity in a number of different experimental models; however the relevance to humans remains poorly understood in most cases (e.g., Li et al. 2013a; Ho and Dargan 2017). On the other hand, urotoxicity and cystitis have been extensively documented among heavy recreational ketamine users (Chu et al. 2007; Shahani et al. 2007; Shahani and Stewart 2008; Tsai et al. 2009; Morgan and Curran 2012). While most cases reported involve nonmedical use and appear to be positively associated with dose and frequency of use, ketamine-induced cystitis has also been documented with medical use for example in patients using ketamine for pain (Storr and Quibell 2009). Ketamine-induced cystitis has been investigated in animal and in vitro models although the pathogenesis remains incompletely understood (Tan et al. 2011; Tang et al. 2015). The toxicology of ketamine including clinical as well as in vitro and in vivo studies in nonhuman animals has been reviewed (e.g., Li et al. 2013a; Ho and Dargan 2017).

3.3 Methoxetamine

Methoxetamine (MXE) (Fig. 2) is the β-keto-derivative of the arylcyclohexylamine research chemical 3-MeO-PCE (Wallach and Brandt 2018). The fascinating history of MXE and how it was developed has been described elsewhere (Morris 2011; Morris and Wallach 2014). Briefly, MXE was designed and explored by a UK chemist attempting to develop an improved version of ketamine to treat his phantom limb pain. The main intentions were to increase potency and duration of action of the treatment and in the process to reduce the risk of bladder toxicity seen with ketamine (Morris 2011; Morris and Wallach 2014). This perceived reduction in bladder toxicity has been reported as a motivation for use among some users (Winstock et al. 2016) although the validity of this theory remains to be established. MXE became available for purchase as a research chemical in 2010 and was notified to the EMCDDA shortly afterward in November of the same year (EMCDDA–Europol 2011). At the present time MXE remains a popular research chemical even though it has been placed under international control.

MXE induces mild dissociative effects starting around 5–10 mg and is active via oral and parenteral routes although potency differs with parenteral routes being generally more potent. Reports on ingestion of higher doses are common with strong dissociative effects and hallucinations generally requiring 30–60 mg. MXE appears

to be around two to threefold more potent than ketamine in human subjects, which is consistent with NMDAR receptor binding affinities and results from animal studies (Horsley et al. 2016; Roth et al. 2013; Halberstadt et al. 2016). Durations of effects depend on the route of administration but are consistently of longer duration than ketamine: insufflation (2.4–4 h), oral (3–5 h), and im (2–3 h). Subjective effects that have been described include euphoria, a sense of calm and serenity, distortion or loss of sensory perception, severe dissociation, depersonalization, and loss of consciousness similar to effects of classical hallucinogens, like LSD and psilocybin, and ketamine (Kjellgren and Jonsson 2013). Since the emergence of MXE, extensive research data have been accumulated within the last few years, and a complete review is out of scope of this chapter although representative information and key studies have been included. Review articles on MXE have been published (e.g., Corazza et al. 2013; WHO 2014; Zawilska 2014; Zanda et al. 2016). It is worth noting that bromo-MXE (Fig. 2) was detected in August 2013 in a sample containing MXE in Europe (EMCDDA–Europol 2014), which indicated that it might have been produced by overbromination during MXE synthesis. No reports on its use or availability could be identified online.

3.3.1 Pharmacokinetics

Following subcutaneous administration of MXE (male Wistar rats, 10 mg/kg), the analysis of brain, liver, and lung tissue at different time points revealed that that highest MXE concentrations in all three tissues were observed within the first hour. Within the first hour, highest concentrations of the *N*-demethyl metabolite nor-MXE were detected in lung tissue, whereas *O*-demethyl-MXE was highest in the liver (Hajkova et al. 2016). An extension of this work confirmed the MXE brain-to-serum ratio to be between 2.06 and 2.93 during the time course of the study. In addition, a longer duration of action, relative to ketamine, was reported, and this was consistent with the time course of detected serum and brain concentrations (Horsley et al. 2016).

A number of studies have investigated metabolism of MXE using in vitro and in vivo methods in rat and humans. Results have been for the most part consistent with ketamine biotransformation and which included *N*- and *O*-dealkylation, hydroxylation of the aryl and aliphatic rings, and detections of dihydro-MXE, dihydronor-MXE, and dihydro-*O*-demethyl-MXE as major phase I metabolites (Meyer et al. 2013a; Menzies et al. 2014; Hajkova et al. 2016; Horsley et al. 2016). In vitro enzyme studies also revealed that CYP2B6 and CYP3A4 isoforms were involved in *N*-deethylation of MXE, whereas CYP2B6 and CYP2C19 were implicated in *O*-demethylation. A comparatively minor formation of the hydroxy-aryl-MXE metabolite was formed during incubation with CYP2B6. Another interesting observation made during the derivatization of metabolite samples for analysis by gas chromatography mass spectrometry was the formation of a cyclized artifact (Meyer et al. 2013a). It should be noted that CYP3A4 and CYP2B6 also play an important role in the metabolism (via *N*-demethylation) of ketamine to its major phase I metabolite norketamine (Yanagihara et al. 2001; Hijazi and Boulieu 2002; Li et al. 2013b). Detected phase II metabolites of MXE include sulfate and

glucuronide metabolites of phase I metabolites (Menzies et al. 2014; Meyer et al. 2013a; Horsley et al. 2016). Nor-MXE (and in one case dihydro-MXE) were found to be the most abundant metabolites in human urine samples (Meyer et al. 2013a; Menzies et al. 2014). In rat urine (male Wistar, sc), the most abundant metabolite (~15%) was *O*-demethyl-MXE followed by nor-MXE (~4%) and dihydro-*O*-demethyl-MXE (~3%) following a subcutaneous injection of 40 mg/kg. The majority of MXE (~75%) was excreted unchanged in urine (Horsley et al. 2016).

3.3.2 Pharmacodynamic Effects In Vitro

MXE showed moderate affinity at the PCP binding site of NMDAR ($K_i = 259$ nM), which was about twofold greater than ketamine ($K_i = 659$ nM) (Roth et al. 2013). This increased affinity is consistent with anecdotal reports of two to threefold greater potency of MXE relative to ketamine in humans and animal studies (Halberstadt et al. 2016). 3-MeO-PCE ($K_i = 61$ nM) had ~4.2-fold higher NMDAR affinity than MXE (Roth et al. 2013) suggesting that the β-keto function decreases NMDAR affinity and potency. Consistent with this, 3-MeO-PCE is about 2-3 fold more potent than MXE via nasal insufflation (personal communication). More structure-activity research is needed to better define the effect of the β-keto substitution on these compounds.

MXE also showed affinity for human SERT (481 nM) but lacked significant affinities ($IC_{50} > 10{,}000$ nM) at human NET and DAT as well as sigma-1 and sigma-2 receptors (Roth et al. 2013). Consistent with the receptor binding studies, MXE was found to block uptake of monoamines at human DAT ($IC_{50} = 33$ μM), NET ($IC_{50} = 20$ μM) and most strongly for SERT ($IC_{50} = 2{,}400$ nM and 2,000 nM) in transfected HEK293 cells (Zwartsen et al. 2017; Hondebrink et al. 2017). MXE inhibited neuronal (electrical) activity ($IC_{50} = 500$ nM) (predominantly characterized as displaying excitatory glutamatergic neurons, inhibitory GABAergic neurons, and astrocytes) using a multi-well microelectrode array. Under these conditions, neuronal activity and modulation represents a measurable integrated output, thus, detailed information about mechanistic features is unavailable. The inhibition of neuronal activity has also been observed with other test substances such as GABA and diazepam (Hondebrink et al. 2016). In a follow-up study, MXE as well as ketamine were evaluated in rat primary cortical cells ($IC_{50} = 500$ and 1,200 nM, respectively), human SH-SY5Y cells, human induced pluripotent stem cell (hiPSC)-derived iCell® neurons, DopaNeurons and astrocyte co-cultures. A number of conditions were investigated, but, in summary, MXE inhibited neuronal activity in rat cortical cultures and iPSC-derived neurons, inhibited voltage-gated Ca^{2+} channels in SH-SY5 cells, and increased glutamate-evoked increase in intracellular Ca^{2+} in rat cortical cultures (Hondebrink et al. 2017).

3.3.3 Effects In Vivo

The available data currently suggest that MXE displays rewarding and reinforcing effects and thus potential abuse liability especially in predisposed individuals. In adult male Sprague-Dawley rats, electrophysiological investigations uncovered that MXE dose dependently stimulated firing and burst rate of ventral tegmental area

dopamine neurons projecting to the nucleus accumbens (NAc) shell after injection of cumulative doses of MXE (0.031–0.5 mg/kg, iv). Correspondingly, and consistent with observations reported with ketamine and PCP, MXE increased extracellular dopamine levels in the NAc shell (0.125, 0.25 and 0.5 mg/kg, iv) using microdialysis studies in freely moving male Sprague-Dawley rats. MXE was also found to substitute for ketamine in a self-administration paradigm in male Sprague-Dawley rats (Mutti et al. 2016). Furthermore, MXE was reported to produce conditioned place preference (2.5 and 5 mg/kg) and to be self-administered in male Sprague-Dawley rats (0.25, 0.5, 1.0 mg/kg, iv infusion). The self-administration was reported to be modest relative to that seen with ketamine (0.5 mg/kg) (Botanas et al. 2015). MXE generalized to ketamine in discriminative stimulus model in male Sprague-Dawley rats (Chiamulera et al. 2016). Interestingly, the highest dose of LSD (0.3 mg/kg) tested also showed (77%) generalization to ketamine (all lower doses tested also showed some generalization). In male Sprague-Dawley rats, MXE (ED_{50} = 0.25 mg/kg) substituted for 3 mg/kg PCP (ED_{50} = 1.25 mg/kg) in a discriminative stimulus test. However substitution was not seen in all animals tested and there was also a reduction in response rates. In addition, MXE dose-dependently reduced PCP withdrawal symptoms and was self-administered (albeit to a lesser extent than PCP) in these rats. MXE also elicited dose-dependent (10–56 mg/kg) hypothermic effects on male NIH Swiss mice (Berquist et al. 2017).

Interestingly, increased locomotor activity in rodents has been observed under some experimental conditions (Halberstadt et al. 2016 (10 mg/kg, sc); Horsley et al. 2016 (5 and 10 mg/kg, sc)) but not in others (Berquist et al. 2017, 1–30 mg/kg, ip; Botanas et al. 2015 (1.25–5 mg/kg, ip)), which, in part, might have been related to dose, species, and strain differences. Higher doses (40 mg/kg) reportedly caused sedation and hypolocomotion in male Wistar rats (Horsley et al. 2016). Another recent study in male Sprague-Dawley rats evaluated a number of behavioral effects of MXE following ip administration. MXE affected spontaneous motor activity dose and time dependently where lower doses (0.5 mg/kg) were associated with hypermotility, whereas higher doses (2.5–5 mg/kg) were generally associated with hypomotility at various time points after administration. In addition to these effects, 5 mg/kg MXE induced transient analgesia in the tail-flick and hot-plate test and increased swimming activity in the forced swim test (Zanda et al. 2017). As stated previously, the forced swim test is a model used for assessing drugs for potential antidepressant activity in humans. MXE (2.5, 5, or 10 mg/kg, ip) was claimed to have "rapid and sustained antidepressant effects" in several models predictive of antidepressant activity in humans including the forced swim test, tail suspension test, and sucrose preference test in male ICR mice. These effects were suggested to be mediated through glutamatergic and serotoninergic mechanisms based on RT-PCR and pharmacological antagonism experiments (Botanas et al. 2017).

Consistent with PCP, ketamine, and other NMDAR antagonists, MXE (sc) caused dose-dependent disruption of PPI (ED_{50} = 1.89 mg/kg) in male Sprague-Dawley and Wistar rats (Halberstadt et al. 2016; Horsley et al. 2016). The effect on PPI was more potent than those recorded for (*S*)-ketamine

(ED_{50} = 2.86 mg/kg) and (*R*)-ketamine (ED_{50} = 6.33 mg/kg) but less than PCP (ED_{50} = 0.88 mg/kg) consistent with NMDAR affinities (Halberstadt et al. 2016).

Histological investigations following daily MXE administration (30 mg/kg, ip) for a period of 3 months were shown to induce statistically significant bladder and kidney toxicity in male ICR mice (Dargan et al. 2014). Comparable effects have been described with ketamine at (30 mg/kg, ip) (Yeung et al. 2009; Tan et al. 2011). Furthermore, chronic treatment (30 mg/kg daily for 4 or 12 weeks) caused inflammation and dysfunction in female Sprague-Dawley rat bladder and cytotoxic and pro-inflammatory effects in human urothelial cells (SV-HUC-1) similar to what has been shown with ketamine (Wang et al. 2017).

3.3.4 Clinical Toxicology

Case reports describing a range of clinical features associated with MXE intoxication and adverse effects have been abundantly published, and a complete overview is outside the scope of this chapter. From this perspective, a number of representative examples from the literature will be presented, and further information may be obtained from more extensive reviews (e.g., EMCDDA 2014; WHO 2014; Zawilska 2014; Zanda et al. 2016). Many factors influence drug use, but it should be noted that examples exist in the case report literature where MXE use was motivated by attempts to self-medicate for various reasons, such as reduction of high-dose codeine intoxication (Sein Anand et al. 2012), chronic foot pain (Maskell et al. 2016), and post-traumatic stress disorder (Striebel et al. 2017).

What appeared to be among the first reports on the acute toxicity of MXE involved a 32-year-old male who was described as initially agitated and in a dissociative state following self-reported intramuscular administration. Clinical signs included tachycardia (105 bpm), hypertension (140/95), mydriasis with pupils reactive, and bilateral rotatory nystagmus. Respiration rate (16), blood glucose (122 mg/dL), and oxygen saturation (98%) were normal. The patient returned to "baseline mental status" within 8 h. Although a powdered sample was shown to consist of MXE, analytical confirmation from biofluids was unavailable (Ward et al. 2011).

Ketamine-like effects were reported in a 19-year-old male with a history of drug use and psychiatric disorders who was receiving treatment with bupropion, aripiprazole, and chlorprothixene. Following an iv injection of an unknown amount of MXE, the patient was admitted to the hospital with extreme agitation, ataxia, and a semistuporous state. Consistent with other reports, the clinical features described included tachycardia, hypertension, confusion, agitation, stupor, ataxia, mydriasis, and nystagmus (Hofer et al. 2012).

Three cases (males aged 42, 29, and 28 years) involving MXE intoxication were associated with ketamine-type dissociative/catatonic effects but also acute sympathomimetic toxicity. Case 1 involved a 42-year-old male found "collapsed" in the street. He was noted to be drowsy (Glasgow Coma Score 6/15), tachycardic (135 bpm), hypertensive (187/83 mmHg), and pyrexial (38.2°C). The serum concentration of MXE detected was 120 ng/mL. The NPS stimulant and sympathomimetic drug 5/6-APB (isomer not determined) was also detected but not quantified

and the patient reported ingesting 3 pints of beer. Case 2 involved a 29-year-old male found "catatonic" by his mother. He had tremor, visual hallucinations, confusion, and mydriasis. At the ER he was found to be confused, tachycardic (121 bpm), and hypertensive (201/104 mmHg). The MXE serum concentration was 90 ng/mL. Diphenhydramine and venlafaxine were also detected. The third case involved a 28-year-old male who had collapsed in the bathroom of a nightclub. He developed worsening agitation and aggression en route to the emergency department. He was tachycardic (113 bpm) and hypertensive (198/78 mmHg) and had mydriasis. The serum concentration of MXE detected was 200 ng/mL (Wood et al. 2012). Subsequently, three further cases (males aged 19, 18, and 18 years) have been described that included clinical features of severe truncal ataxia, nystagmus, incoordination, and reduced conscious level several hours after nasal insufflation. Features of cerebellar toxicity persisted 3–4 days in one case (19-year-old male). Slurred speech, incoordination, and cerebellar ataxia were also noted in addition to sympathomimetic features (Shields et al. 2012).

What appears to be one of the earliest accidental fatal intoxications involving MXE was reported in 2013 in which the MXE concentration found in femoral blood was 8,600 ng/g. Therapeutic concentrations of venlafaxine (300 ng/g) and *O*-demethylvenlafaxine (400 ng/g) were detected in addition to tetrahydrocannabinol (1 ng/g). Three synthetic cannabinoid receptor agonists (AM-694, AM-2201, and JWH-018) were also detected (<1 ng/g). These other substances may have contributed to the fatal outcome (Wikstrom et al. 2013). An estimated blood concentration of 5,800 ng/mL (urine: 85,000 ng/mL) MXE has been reported in another case of fatal intoxication in a 29-year-old male (Adamowicz and Zuba 2015).

Chronic recreational ketamine use has been associated with bladder toxicity (e.g., ulcerative cystitis) (Morgan and Curran 2012), and further studies are needed to assess the extent to which this might also apply to MXE. As stated previously, nonhuman animal and in vitro studies suggest it to be a possibility especially with higher doses and frequent use (Dargan et al. 2014; Wang et al. 2017). Furthermore, a survey carried out in 2012 including respondents who reported having ever used ketamine and MXE in the past 12 months revealed that 23% reported experiencing urinary symptoms (e.g., frequent urination, or pain in lower abdomen, etc.) although previous ketamine use could not be ruled out as a causative or contributing factor (Lawn et al. 2016).

3.4 Deschloroketamine (DCK)

Since 2015, a drug testing service coordinated by the drug information center Erowid disseminated information about the detection of deschloroketamine (2-oxo-PCMe, DCK) in powdered samples originating from the USA, China, and Europe. In addition, some of the samples were mislabeled (EcstasyData.org 2018). The detection of DCK has also been reported in 2015 in Barcelona (Spain) (Energycontrol 2015). Two samples recently sold as 2-oxo-PCMe were

subsequently found to be 2-oxo-PCE based on analysis by GC-MS (Wallach and Morris unpublished). DCK is available from online vendors (Van Hout and Hearne 2017). A patent describing the synthesis of DCK was filed in 1962 (Stevens 1962), followed by various publications afterward (Stevens et al. 1963, 1966a, b; Preiss and Tatar 1995; Brunner et al. 2003) including descriptions published in the public domain (Anonymous 2007). The synthetic preparation of DCK has also been featured on a TV program (Viceland 2017). DCK was first reported to the EMCDDA in March 2015 (EMCDDA–Europol 2016), and analytical data have been published (Frison et al. 2016; Maixner et al. 2017) including X-ray powder diffraction analysis on the (*S*)-enantiomer (Maixner et al. 2017). Some analytical information is also available in the public domain (Hungarian Institute for Forensic Science 2016). DCK is currently a fairly popular research chemical with a great deal of discussion on online forums touting its desirable activity profile. DCK was found to be a potent dissociative agent able to induce ethanol-like dissociative effects beginning with doses as low as 4 mg (nasal insufflation of the HCl salt) although higher doses induce more potent dissociative effects (personal communication). Users on Internet forums report a range of doses and routes of administration. The use of DCK for the treatment of bacterial, fungal, viral, or protozoal infections and for immunomodulation has been described. Claimed clinical examples included treatment of cerebral toxoplasmosis, cytomegalovirus infection, conjunctivitis, herpes, and infections associated with HIV (Preiss and Tatar 1995).

3.5 Deschloro-*N*-Ethyl-Norketamine (2-Oxo-PCE)

2-oxo-PCE was apparently the first β-keto-arylcyclohexylamine evaluated by Parke-Davis from the lab of Calvin Stevens. Based on the positive results observed with 2-oxo-PCE, Stevens and his group synthesized and submitted a number of related compounds to Parke-Davis for further testing which led to the identification of ketamine (McCarthy 1981). The synthesis of 2-oxo-PCE was included in the same patent used for the disclosure of the DCK procedure (Stevens 1962), followed by additional examples (Stevens et al. 1966a, 1972). Samples of 2-oxo-PCE have been identified in powdered samples including examples where they were sold as DCK (EcstasyData.org 2018). 2-oxo-PCE induced dissociative effects and strong analgesic effects at 4 mg (nasal insufflation of HCl salt) with an ~3 h duration. Higher doses (17 mg via nasal insufflation) were reported to induce strong dissociatives effects that were said to be "equal to 80 mg of ketamine" (personal communication). The first detection of 2-oxo-PCE has been reported to the EMCDDA in August 2016 (EMCDDA–Europol 2017). Some analytical information is also available in the public domain (Slovenian National Forensic Laboratory 2016). The related *N*-propyl analog 2-oxo-PCPr is a potent dissociative compound capable of inducing ethanol-like effects beginning around 3 mg (nasal insufflation of the HCl salt) (personal communication); however it does not appear to have been sold as a research chemical to date.

3.6 Other β-Keto-Arylcyclohexylamines

A number of additional β-keto-arylcyclohexylamines continue to be offered for sale on the NPS market (Fig. 2). Some reports of dissociative effects with these compounds can be found on numerous online discussion forms (e.g., reddit.com, bluelight.ru, drugs-forum.com). As described in the previous cases, the origin of many of these compounds can be traced back to the scientific literature although a few appear to be novel creations (FXE). The available data on these compounds are currently limited but some are commercially available as reference material.

3.6.1 *N*-Ethylnorketamine (NEK)

The structure of NEK was captured in a patent published by Stevens (e.g., 1962). Some information on the effects of NEK emerged in 2010 followed by increasing discussions on user forums when it emerged into the public space in 2012 (Morris and Wallach 2014). The first detection of NEK was reported to the EMCDDA in September 2012 (EMCDDA–Europol 2013). A published report exists about its detection from samples obtained from uncompleted postal deliveries to Northern Ireland (Jones et al. 2016).

3.6.2 2-Methoxy-2-Deschloroketamine (2-MK)

The synthesis of 2-MK (dinoket) was described in a patent published by Stevens (e.g., 1962) as well as in research article format (Stevens et al. 1966c). Similar to NEK, the interest in 2-MK as a research chemical originated in 2010 when first claims about its psychoactive properties became openly available. However, reports indicated that its effects in users might have been considered disappointing when it became available as a research chemical in 2012 in part due to low potency (Morris and Wallach 2014). 2-MK was first reported to the EMCDDA in August 2012 (EMCDDA–Europol 2013). Its detection has since been reported in Sweden (Backberg et al. 2018), and a conversion of racemic 2-MK into the corresponding diastereomers with trifluoroacetyl-L-prolyl chloride followed by GC-MS analysis for analytical purposes has been published (Weiß et al. 2015).

3.6.3 Methoxmetamine (MXM)

MXM has been described as an active dissociative with reduced potency and shorter duration than MXE with 50–100 mg doses described as active (oral and parenteral) by users on online discussion forms although reports of higher doses exist. This is consistent with the difference in potency and NMDAR binding affinity between 3-MeO-PCMe and 3-MeO-PCE (Wallach and Brandt 2018). Reports of MXM as a research chemical started to appear online in 2014 (Anonymous 2014). A test purchase from an online research chemical vendor was tentatively confirmed by GC-MS and nuclear magnetic resonance spectroscopy (NMR) (Wallach unpublished). The identification of a MXM sample seized in Japan (termed MMXE) and its analytical characterization have been described (Kaizaki-Mitsumoto et al. 2016).

3.6.4 2-Fluoro-2-Deschloroketamine (2-FDCK)

The synthesis of 2-FDCK has been reported (Wang and Li 1987; Moghimi et al. 2014), and discussions about 2-FDCK on online drug discussion forms started to surface in 2015 (e.g., Anonymous 2015a, 2017). 2-FDCK was offered for sale by online research chemical vendors around this time. Information about the analysis of powdered samples has been disseminated including one sample that has apparently been sold as ketamine (EcstasyData.org 2018). Internet discussions suggest that 2-FDCK might be an active dissociative drug with potency comparable (or slightly more so) than ketamine. The analysis of a sample obtained from a test purchase (GC-MS and NMR) appeared consistent with the described structure (Wallach unpublished). Insufflation of 50 mg of analytically confirmed FDCK (salt unknown but suspected HCl, consumed over about an hour) induced dissociative effects with fluctuating tinnitus (personal communication). Although detailed pharmacological data on 2-FDCK could not be identified, Moghimi et al. (2014) stated that "preliminary animal tests on mice have shown that the resulting fluoroketamine has some advantages over ketamine in terms of the effective dose and the recovery time." However, further details were not included. A few other fluorinated β-keto-arylcyclohexylamines can be found discussed on online drug discussion forms including fluorexetamine (FXE) and 2-trifluoromethyl-2-deschloroketamine (2TFMDCK) (Fig. 2). More research is needed on these compounds.

4 Conclusions

The currently available data suggest that the 1,2-diarylethylamine- and β-keto-arylcyclohexylamine-based NPS show high to moderate affinities for the NMDAR where they also act as uncompetitive antagonists. In some cases, NMDAR affinity appears to correlate well with the dissociative properties in humans, whereas the 1,2-diarylethylamines evaluated show reduced potency in humans and animal models relative to their NMDAR affinity. The cause for this is unknown and warrants additional investigations although pharmacokinetic variables may be important. Furthermore, some non-NMDAR receptor interactions have been noted including affinities for and inhibition of monoamine reuptake transporters, as well as affinities for sigma receptors and in few cases also opioid receptor and α-adrenergic subtypes. The contribution of these mechanisms to the subjective effects of these compounds remains poorly understood, but it is possible they can contribute to the activity of individual compounds. Common clinical features reported from acute intoxication cases have included confusion, hallucination, dissociation, catatonia, euphoria, comatose states, and nystagmus but also hypertension and tachycardia. Other compounds that act through NMDAR known to cause dissociative effects are available from Internet retailers (e.g., memantine and D-cycloserine) but detailed information about their use and popularity is currently limited. Evolution of legislative control measures, market demand, and technology is likely to continue to change the research chemical market. Compared to other NPS classes, such as the synthetic cathinones or cannabinoid receptor agonists, the number of dissociative

drugs available on the open NPS market is comparatively small, but the potential for expanding the product catalogs is significant, which includes potential for research on drugs with potential clinical utility.

References

Aalto S, Hirvonen J, Kajander J, Scheinin H, Någren K, Vilkman H, Gustafsson L, Syvälahti E, Hietala J (2002) Ketamine does not decrease striatal dopamine D_2 receptor binding in man. Psychopharmacology 164:401–406

Abel KM, Allin MP, Hemsley DR, Geyer MA (2003) Low dose ketamine increases prepulse inhibition in healthy men. Neuropharmacology 44:729–737

Adamowicz P, Zuba D (2015) Fatal intoxication with methoxetamine. J Forensic Sci 60(Suppl 1): S264–S268

Adams JD Jr, Baillie TA, Trevor AJ, Castagnoli N Jr (1981) Studies on the biotransformation of ketamine. 1. Identification of metabolites produced in vitro from rat liver microsomal preparations. Biomed Mass Spectrom 8:527–538

Alvarez J-C, Fabresse N, Knapp A, El Hajj Sleiman I, Garnier R, Langrand J (2017) Identification and quantification of diphenidine in hair by LC-MS/MS after single administration. Toxicol Anal Clin 29:64–70

Amphoux A, Vialou V, Drescher E, Brüss M, Mannoury La Cour C, Rochat C, Millan MJ, Giros B, Bönisch H, Gautron S (2006) Differential pharmacological in vitro properties of organic cation transporters and regional distribution in rat brain. Neuropharmacology 50:941–952

Anis NA, Berry SC, Burton NR, Lodge D (1983) The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurons by N-methyl-aspartate. Br J Pharmacol 79:565–575

Anonymous (1960) Pharmaceutical compositions and methods for producing phenylcyclohexane compounds. Patent No. GB853775A. Parke Davis, Detroit

Anonymous (2007) Synthese von 2-Methylamino-2-phenylcyclohexanon (MPCH) [7063-30-1]. Last updated: 11 May 2007. http://www.lambdasyn.org/synfiles/mpch.htm. Accessed 26 Mar 2018

Anonymous (2014) The Big & Dandy Methoxmetamine/MXM Thread - V1: Note, this is not MXE! Bluelight. http://www.bluelight.org/vb/threads/732888-The-Big-amp-Dandy-Methoxmetamine-MXM-Thread-V1-Note-this-is-not-MXE! Accessed 24 Apr 2018

Anonymous (2015a) The Big & Dandy 2-Fluoroketamine Thread. Bluelight. http://www.bluelight.org/vb/threads/776753-The-Big-amp-Dandy-2-Fluoroketamine-Thread. Accessed 24 Apr 2018

Anonymous (2015b) Fluorolintane. https://www.ukchemicalresearch.org/Thread-Fluorolintane. Accessed 3 Feb 2018

Anonymous (2017) 2f-DCK vs DCK vs O-PCE, what do you prefer? Reddit. https://www.reddit.com/r/researchchemicals/comments/75iydc/2fdck_vs_dck_vs_opce_what_do_you_prefer/. Accessed 3 Feb 2018

Aspergren BD, Heinzelman RV (1963) Therapeutic 1-(1,2-diphenylethyl) pyrrolidine for the management of depression. Patent No. US3083139. Upjohn Company, Kalamazoo

Aspergren BD, Heinzelman RV (1964) Process for obtaining weight reduction. Patent No. US3134716. Upjohn Company, Kalamazoo

Azzaro AJ, Smith DJ (1977) The inhibitory action of ketamine HCl on [^{3}H]5-hydroxytryptamine accumulation by rat brain synaptosomal-rich fractions: comparison with [^{3}H]catecholamine and [^{3}H]γ-aminobutyric acid uptake. Neuropharmacology 16:349–356

Backberg M, Jonsson KH, Beck O, Helander A (2018) Investigation of drug products received for analysis in the Swedish STRIDA project on new psychoactive substances. Drug Test Anal 10:340–349

Beharry S, Gibbons S (2016) An overview of emerging and new psychoactive substances in the United Kingdom. Forensic Sci Int 267:25–34

Berger ML, Schweifer A, Rebernik P, Hammerschmidt F (2009) NMDA receptor affinities of 1,2-diphenylethylamine and 1-(1,2-diphenylethyl)piperidine enantiomers and of related compounds. Bioorg Med Chem 17:3456–3462

Berquist MD, Hyatt WS, Bauer-Erickson J, Gannon BM, Norwood AP, Fantegrossi WE (2017) Phencyclidine-like in vivo effects of methoxetamine in mice and rats. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2017.1008.1028

Boateng BO, Fever M, Edwards D, Petersson P, Euerby MR, Sutcliffe OB (2018) Chromatographic retention behaviour, modelling and optimization of a UHPLC-UV separation of the regioisomers of the Novel Psychoactive Substance (NPS) methoxphenidine (MXP). J Pharm Biomed Anal 153:238–247

Botanas CJ, de la Peña JB, Dela Peña IJ, Tampus R, Yoon R, Kim HJ, Lee YS, Jang CG, Cheong JH (2015) Methoxetamine, a ketamine derivative, produced conditioned place preference and was self-administered by rats: evidence of its abuse potential. Pharmacol Biochem Behav 133:31–36

Botanas CJ, Bryan de la Pena J, Custodio RJ, Joy Dela Pena I, Kim M, Woo T, Kim HJ, Kim HI, Chang Cho M, Lee YS, Cheong JH (2017) Methoxetamine produces rapid and sustained antidepressant effects probably via glutamatergic and serotonergic mechanisms. Neuropharmacology 126:121–127

Brunner H, Kagan HB, Kreutzer G (2003) Asymmetric catalysis. Part 153: metal-catalysed enantioselective α-ketol rearrangement. Tetrahedron Asymmetry 14:2177–2187

Caloro M, Calabro G, de Pisa E, Rosini E, Kotzalidis GD, Lonati D, Locatelli CA, Papa P, Schifano F, Girardi P (2018) Combined NMDA inhibitor use in a patient with multisubstance-induced psychotic disorder. J Addict Med. https://doi.org/10.1097/adm.0000000000000390

Campbell KN, Helbing CH, Florkowski MP, Campbell BK (1948) The reaction of Grignard reagents with Schiff bases. J Am Chem Soc 70:3868–3870

Can A, Zanos P, Moaddel R, Kang HJ, Dossou KS, Wainer IW, Cheer JF, Frost DO, Huang XP, Gould TD (2016) Effects of ketamine and ketamine metabolites on evoked striatal dopamine release, dopamine receptors, and monoamine transporters. J Pharmacol Exp Ther 359:159–170

Chambers RA, Bremner JD, Moghaddam B, Southwick SM, Charney DS, Krystal JH (1999) Glutamate and post-traumatic stress disorder: toward a psychobiology of dissociation. Semin Clin Neuropsychiatry 4:274–281

Champeau W, Eiden C, Gambier J, Peyriere H (2017) Methoxphenidine use disorder: first case notified to the French addictovigilance network. J Clin Psychopharmacol 37:376–377

Chen G (1969) The pharmacology of ketamine. In: Kreuscher H (ed) Ketamine: Bericht über das internationale Symposion am 23. und 24. Februar 1968 in Mainz. Springer, Berlin, Heidelberg, pp 1–11

Chen X, Shu S, Bayliss DA (2009) HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. J Neurosci 29:600–609

Cheng HC, Long JP, Van Orden LS III, Cannon JG, O'Donnell JP (1976) Dopaminergic activity of some apomorphine analogs. Res Commun Chem Pathol Pharmacol 15:89–106

Chiamulera C, Armani F, Mutti A, Fattore L (2016) The ketamine analogue methoxetamine generalizes to ketamine discriminative stimulus in rats. Behav Pharmacol 27:204–210

Chodoff P, Stella JG (1966) Use of CI-581: a phencyclidine derivative for obstetric anesthesia. Anesth Analg 45:527–530

Chretien B, Bourgine J, Hamel Sénécal L, Bretaudeau-Deguigne M, Boels D, Lelong-Boulouard V, Le Boisselier R (2018) Severe serotonin syndrome in an autistic new psychoactive substance user after consumption of pills containing methoxphenidine and α-methyltryptamine. J Clin Psychopharmacol 38:94–96

Christiaen A (1924) Contribution à l'étude de la réaction des organo-magnésiens sur les nitriles. Les nitriles α amines. Bull Soc Chim Belg 33:483–490

Chu PS, Kwok SC, Lam KM, Chu TY, Chan SW, Man CW, Ma WK, Chui KL, Yiu MK, Chan YC, Tse ML, Lau FL (2007) 'Street ketamine'-associated bladder dysfunction: a report of ten cases. Hong Kong Med J 13:311–313

Cilia J, Hatcher P, Reavill C, Jones DN (2007) (±)Ketamine-induced prepulse inhibition deficits of an acoustic startle response in rats are not reversed by antipsychotics. J Psychopharmacol 21:302–311

Clements JA, Nimmo WS (1981) Pharmacokinetics and analgesic effect of ketamine in man. Br J Anaesth 53:27–30

Cohen ML, Trevor AJ (1974) On the cerebral accumulation of ketamine and the relationship between metabolism of the drug and its pharmacological effects. J Pharmacol Exp Ther 189:351–358

Colestock T, Wallach J, Mansi M, Filemban N, Morris H, Elliott SP, Westphal F, Brandt SD, Adejare A (2018) Syntheses, analytical and pharmacological characterizations of the 'legal high' 4-[1-(3-methoxyphenyl)cyclohexyl]morpholine (3-MeO-PCMo) and analogues. Drug Test Anal 10:272–283

Coppola M, Mondola R (2012) Methoxetamine: from drug of abuse to rapid-acting antidepressant. Med Hypotheses 79:504–507

Coppola M, Mondola R (2013) Is methoxydine a new rapid acting antidepressant for the treatment of depression in alcoholics? Med Hypotheses 81:10–14

Corazza O, Assi S, Schifano F (2013) From "Special K" to "Special M": the evolution of the recreational use of ketamine and methoxetamine. CNS Neurosci Ther 19:454–460

Corssen G, Domino EF (1966) Dissociative anesthesia: further pharmacologic studies and first clinical experience with the phencyclidine derivative CI-581. Anesth Analg 45:29–40

Critchlow DG (2006) A case of ketamine dependence with discontinuation symptoms. Addiction 101:1212–1213

Cumming JF (1976) The development of an acute tolerance to ketamine. Anesth Analg 55:788–791

Dargan PI, Tang HC, Liang W, Wood DM, Yew DT (2014) Three months of methoxetamine administration is associated with significant bladder and renal toxicity in mice. Clin Toxicol (Phila) 52:176–180

Davies SN, Alford ST, Coan EJ, Lester RA, Collingridge GL (1988) Ketamine blocks an NMDA receptor-mediated component of synaptic transmission in rat hippocampus in a voltage-dependent manner. Neurosci Lett 92:213–217

Dayton PG, Stiller RL, Cook DR, Perel JM (1983) The binding of ketamine to plasma proteins: emphasis on human plasma. Eur J Clin Pharmacol 24:825–831

de Bruin NM, Ellenbroek BA, Cools AR, Coenen AM, van Luijtelaar EL (1999) Differential effects of ketamine on gating of auditory evoked potentials and prepulse inhibition in rats. Psychopharmacology 142:9–17

De Luca MT, Badiani A (2011) Ketamine self-administration in the rat: evidence for a critical role of setting. Psychopharmacology 214:549–556

Dodds EC, Lawson W, Williams PC (1944) Morphine-like properties of diphenylethylamine and related compounds. Proc R Soc Lond B Biol Sci 132:119–132

Dodds EC, Lawson W, Simpson SA, Williams PC (1945) Testing diphenylethylamine compounds for analgesic action. J Physiol 104:47–51

Domino EF (2010) Taming the ketamine tiger. Anesthesiology 113:678–684

Domino EF (ed) (2018) Status of ketamine in anesthesiology [reprint from 1990]. NPP Books, Arlington

Domino EF, Chodoff P, Corssen G (1965) Pharmacologic effects of CI-581, a new dissociative anesthetic, in man. Clin Pharmacol Ther 6:279–291

Ebert B, Mikkelsen S, Thorkildsen C, Borgbjerg FM (1997) Norketamine, the main metabolite of ketamine, is a non-competitive NMDA receptor antagonist in the rat cortex and spinal cord. Eur J Pharmacol 333:99–104

EcstasyData.org (2018) https://www.ecstasydata.org/results.php?start=0&search_field=all&s=deschloroketamine. Accessed 2 Apr 2018

Elliott SP, Brandt SD, Freeman S, Archer RP (2013) AMT (3-(2-aminopropyl)indole) and 5-IT (5-(2-aminopropyl)indole): an analytical challenge and implications for forensic analysis. Drug Test Anal 5:196–202

Elliott SP, Brandt SD, Wallach J, Morris H, Kavanagh PV (2015) First reported fatalities associated with the 'research chemical' 2-methoxydiphenidine. J Anal Toxicol 39:287–293

Elliott S, Sedefov R, Evans-Brown M (2018) Assessing the toxicological significance of new psychoactive substances in fatalities. Drug Test Anal 10:120–126

EMCDDA (2014) Methoxetamine. Report on the risk assessment of 2-(3-methoxyphenyl)-2-(ethylamino)cyclohexanone (methoxetamine) in the framework of the Council Decision on new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/775/TDAK14004ENN_480922.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2011) EMCDDA–Europol 2010 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/644/EMCDDA-Europol_Annual_Report_2010A_281336.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2013) New drugs in Europe, 2012. EMCDDA–Europol 2012 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/734/EMCDDA-Europol_2012_Annual_Report_final_439477.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2014) EMCDDA–Europol 2013 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/814/TDAN14001ENN_475519.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2015) EMCDDA–Europol 2014 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/1018/TDAN15001ENN.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2016) EMCDDA–Europol 2015 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/2880/TDAS16001ENN.pdf. Accessed 3 Feb 2018

EMCDDA–Europol (2017) EMCDDA–Europol 2016 annual report on the implementation of Council Decision 2005/387/JHA. In accordance with Article 10 of Council Decision 2005/387/JHA on the information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon. http://www.emcdda.europa.eu/system/files/publications/4724/TDAN17001ENN_PDFWEB.pdf. Accessed 3 Feb 2018

Energycontrol (2015) Alerta: descloroketamina vendida como ketamina en Barcelona. https://energycontrol.org/analisis-de-sustancias/resultados/alertas/560-alerta-descloroketamina-vendida-como-ketamina-en-barcelona.html. Accessed 3 Feb 2018

Fourcade EW, Lapidus KAB (2016) The basic and clinical pharmacology of ketamine. In: Mathew SJ, Zarate JCA (eds) Ketamine for treatment-resistant depression: the first decade of progress. Springer, Cham, pp 13–29. https://doi.org/10.1007/978-3-319-42925-0_2

Frankiewicz T, Potier B, Bashir ZI, Collingridge GL, Parsons CG (1996) Effects of memantine and MK-801 on NMDA-induced currents in cultured neurones and on synaptic transmission and LTP in area CA1 of rat hippocampal slices. Br J Pharmacol 117:689–697

Fray MJ, Bish G, Brown AD, Fish PV, Stobie A, Wakenhut F, Whitlock GA (2006a) N-(1,2-Diphenylethyl)piperazines: a new class of dual serotonin/noradrenaline reuptake inhibitor. Bioorg Med Chem Lett 16:4345–4348

Fray MJ, Bish G, Fish PV, Stobie A, Wakenhut F, Whitlock GA (2006b) Structure-activity relationships of N-substituted piperazine amine reuptake inhibitors. Bioorg Med Chem Lett 16:4349–4353

Frison G, Zamengo L, Zancanaro F, Tisato F, Traldi P (2016) Characterization of the designer drug deschloroketamine (2-methylamino-2-phenylcyclohexanone) by gas chromatography/mass spectrometry, liquid chromatography/high-resolution mass spectrometry, multistage mass spectrometry, and nuclear magnetic resonance. Rapid Commun Mass Spectrom 30:151–160

Frohlich J, Van Horn JD (2014) Reviewing the ketamine model for schizophrenia. J Psychopharmacol 28:287–302

Garcia Ruano JL, Parra A, Aleman J, Yuste F, Mastranzo VM (2009) Monoalkylation of primary amines and N-sulfinylamides. Chem Commun 404–406

Garcia LS, Comim CM, Valvassori SS, Reus GZ, Barbosa LM, Andreazza AC, Stertz L, Fries GR, Gavioli EC, Kapczinski F, Quevedo J (2008) Acute administration of ketamine induces antidepressant-like effects in the forced swimming test and increases BDNF levels in the rat hippocampus. Prog Neuro-Psychopharmacol Biol Psychiatry 32:140–144

Gerace E, Bovetto E, Corcia DD, Vincenti M, Salomone A (2017) A case of nonfatal intoxication associated with the recreational use of diphenidine. J Forensic Sci 62:1107–1111

Geyer PM, Hulme MC, Irving JPB, Thompson PD, Ashton RN, Lee RJ, Johnson L, Marron J, Banks CE, Sutcliffe OB (2016) Guilty by dissociation – development of gas chromatography–mass spectrometry (GC-MS) and other rapid screening methods for the analysis of 13 diphenidine-derived new psychoactive substances (NPSs). Anal Bioanal Chem 408:8467–8481

Ghosh P, Bolt AG, Mrongovius RI (1978) 1,2-Diphenylethylamines as potential non-stimulant anorectics. Arzneimittelforschung 28:1561–1564

Goodson LH, Christopher H (1950) Diphenylethylamines.I. The preparation of tertiary amines by the Grignard reaction. J Am Chem Soc 72:358–362

Goodson LH, Wiegand CJW, Splitter JS (1946) Analgesics. I. N-Alkylated 1,2-diphenylethylamines prepared by the Leuckart reaction. J Am Chem Soc 68:2174–2175

Gouzoulis-Mayfrank E, Heekeren K, Neukirch A, Stoll M, Stock C, Obradovic M, Kovar KA (2005) Psychological effects of (S)-ketamine and N,N-dimethyltryptamine (DMT): a double-blind, cross-over study in healthy volunteers. Pharmacopsychiatry 38:301–311

Grant IS, Nimmo WS, Clements JA (1981) Pharmacokinetics and analgesic effects of i.m. and oral ketamine. Br J Anaesth 53:805–810

Gray NM, Cheng BK (1989) 1,2-Diarylethylamines for treatment of neurotoxic injury. Patent No. EP346791A1. G.D. Searle and Co., Chicago

Grumann C, Vogt S, Huppertz LM, Moosmann B, Franz F, Angerer V, Kramer L, Auwärter V (2016) Drowning due to an intoxication involving the designer drug methoxphenidine – a case report. In: Poster. 54th annual meeting of TIAFT, Brisbane, 28 Aug–1 Sept. https://www.uniklinik-freiburg.de/fileadmin/mediapool/08_institute/rechtsmedizin/pdf/Poster_2016/Grumann_-_Tiaft_2016.pdf. Accessed 3 Feb 2018

Hajkova K, Jurasek B, Sykora D, Palenicek T, Miksatkova P, Kuchar M (2016) Salting-out-assisted liquid-liquid extraction as a suitable approach for determination of methoxetamine in large sets of tissue samples. Anal Bioanal Chem 408:1171–1181

Halberstadt AL, Slepak N, Hyun J, Buell MR, Powell SB (2016) The novel ketamine analog methoxetamine produces dissociative-like behavioral effects in rodents. Psychopharmacology 233:1215–1225

Hansch C, Bjorkroth JP, Leo A (1987) Hydrophobicity and central nervous system agents: on the principle of minimal hydrophobicity in drug design. J Pharm Sci 76:663–687

Harvey M, Sleigh J, Voss L, Jose J, Gamage S, Pruijn F, Liyanage S, Denny W (2015) Development of rapidly metabolized and ultra-short-acting ketamine analogs. Anesth Analg 121:925–933

Hasegawa K, Wurita A, Minakata K, Gonmori K, Nozawa H, Yamagishi I, Watanabe K, Suzuki O (2015) Postmortem distribution of AB-CHMINACA, 5-fluoro-AMB, and diphenidine in body fluids and solid tissues in a fatal poisoning case: usefulness of adipose tissue for detection of the drugs in unchanged forms. Forensic Toxicol 33:45–53

Hearne E, Van Hout MC (2016) "Trip-sitting" in the black hole: a netnographic study of dissociation and indigenous harm reduction. J Psychoactive Drugs 48:233–242

Heekeren K, Neukirch A, Daumann J, Stoll M, Obradovic M, Kovar KA, Geyer MA, Gouzoulis-Mayfrank E (2007) Prepulse inhibition of the startle reflex and its attentional modulation in the human S-ketamine and N,N-dimethyltryptamine (DMT) models of psychosis. J Psychopharmacol 21:312–320

Heinzelman RV, Aspergren BD (1953) Compounds containing the pyrrolidine ring. Analogs of sympathomimetic amines. J Am Chem Soc 75:3409–3413

Helander A, Beck O, Bäckberg M (2015) Intoxications by the dissociative new psychoactive substances diphenidine and methoxphenidine. Clin Toxicol (Phila) 53:446–453

Hesp KD, Stradiotto M (2010) Stereo- and regioselective gold-catalyzed hydroamination of internal alkynes with dialkylamines. J Am Chem Soc 132:18026–18029

Hijazi Y, Boulieu R (2002) Contribution of CYP3A4, CYP2B6, and CYP2C9 isoforms to N-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 30:853–858

Hirota K, Lambert DG (1996) Ketamine: its mechanism(s) of action and unusual clinical uses. Br J Anaesth 77:441–444

Hirota K, Okawa H, Appadu BL, Grandy DK, Devi LA, Lambert DG (1999) Stereoselective interaction of ketamine with recombinant μ, κ, and δ opioid receptors expressed in Chinese hamster ovary cells. Anesthesiology 90:174–182

Ho JH, Dargan PI (2017) Arylcyclohexamines: ketamine, phencyclidine, and analogues. In: Brent J, Burkhart K, Dargan P et al (eds) Critical care toxicology: diagnosis and management of the critically poisoned patient. Springer, Cham, pp 1439–1484

Hofer KE, Grager B, Müller DM, Rauber-Lüthy C, Kupferschmidt H, Rentsch KM, Ceschi A (2012) Ketamine-like effects after recreational use of methoxetamine. Ann Emerg Med 60:97–99

Hofer KE, Degrandi C, Müller DM, Zürrer-Härdi U, Wahl S, Rauber-Lüthy C, Ceschi A (2014) Acute toxicity associated with the recreational use of the novel dissociative psychoactive substance methoxphenidine. Clin Toxicol 52:1288–1291

Holtman JR Jr, Crooks PA, Johnson-Hardy JK, Hojomat M, Kleven M, Wala EP (2008) Effects of norketamine enantiomers in rodent models of persistent pain. Pharmacol Biochem Behav 90:676–685

Hondebrink L, Verboven AHA, Drega WS, Schmeink S, de Groot M, van Kleef R, Wijnolts FMJ, de Groot A, Meulenbelt J, Westerink RHS (2016) Neurotoxicity screening of (illicit) drugs using novel methods for analysis of microelectrode array (MEA) recordings. Neurotoxicology 55:1–9

Hondebrink L, Kasteel EEJ, Tukker AM, Wijnolts FMJ, Verboven AHA, Westerink RHS (2017) Neuropharmacological characterization of the new psychoactive substance methoxetamine. Neuropharmacology 123:1–9

Hondebrink L, Zwartsen A, Westerink RHS (2018) Effect fingerprinting of new psychoactive substances (NPS): what can we learn from in vitro data? Pharmacol Ther 182:193–224

Horsley RR, Lhotkova E, Hajkova K, Jurasek B, Kuchar M, Palenicek T (2016) Detailed pharmacological evaluation of methoxetamine (MXE), a novel psychoactive ketamine analogue – behavioural, pharmacokinetic and metabolic studies in the Wistar rat. Brain Res Bull 126:102–110

Hungarian Institute for Forensic Science (2016) Analytical data for Deschloroketamine. Hungarian Institute for Forensic Science. https://www.policija.si/apps/nfl_response_web/0_Analytical_Reports_final/Deschloroketamine-ID-HIFS003_rpt.pdf. Accessed 2 Apr 2018

Hurt PH, Ritchie EC (1994) A case of ketamine dependence. Am J Psychiatry 151:779

Hustveit O, Maurset A, Øye I (1995) Interaction of the chiral forms of ketamine with opioid, phencyclidine, sigma and muscarinic receptors. Pharmacol Toxicol 77:355–359

Jansen KLR (2004) Ketamine: dreams and realities. Multidisciplinary Association for Psychedelic Studies, Sarasota. https://www.maps.org/images/pdf/books/K-DreamsKJansenMAPS.pdf. Accessed 8 Feb 2018

Jones LE, Stewart A, Peters KL, McNaul M, Speers SJ, Fletcher NC, Bell SE (2016) Infrared and Raman screening of seized novel psychoactive substances: a large scale study of >200 samples. Analyst 141:902–909

Jordan S, Chen R, Fernalld R, Johnson J, Regardie K, Kambayashi J, Tadori Y, Kitagawa H, Kikuchi T (2006) In vitro biochemical evidence that the psychotomimetics phencyclidine, ketamine and dizocilpine (MK-801) are inactive at cloned human and rat dopamine D_2 receptors. Eur J Pharmacol 540:53–56

Kaizaki-Mitsumoto A, Noguchi N, Yamaguchi S, Odanaka Y, Matsubayashi S, Kumamoto H, Fukuhara K, Funada M, Wada K, Numazawa S (2016) Three 25-NBOMe-type drugs, three other phenethylamine-type drugs (25I-NBMD, RH34, and escaline), eight cathinone derivatives, and a phencyclidine analog MMXE, newly identified in ingredients of drug products before they were sold on the drug market. Forensic Toxicol 34:108–114

Kamenka JM, Geneste P (1981) Synthesis, conformation, and physical properties of phencyclidine and its derivatives. In: Domino EF (ed) PCP (phencyclidine): historical and current perspectives. NPP Books, Ann Arbor, pp 47–82

Kang H, Park P, Bortolotto ZA, Brandt SD, Colestock T, Wallach J, Collingridge GL, Lodge D (2017) Ephenidine: a new psychoactive agent with ketamine-like NMDA receptor antagonist properties. Neuropharmacology 112:144–149

Kapur S, Seeman P (2002) NMDA receptor antagonists ketamine and PCP have direct effects on the dopamine D_2 and serotonin 5-HT_2 receptors-implications for models of schizophrenia. Mol Psychiatry 7:837–844

Kasé Y, Yuizono T, Muto M (1963) Piperidino groups in antitussive activity. J Med Chem 6:118–122

Katalinic N, Lai R, Somogyi A, Mitchell PB, Glue P, Loo CK (2013) Ketamine as a new treatment for depression: a review of its efficacy and adverse effects. Aust N Z J Psychiatry 47:710–727

Keiser M, Hasan M, Oswald S (2018) Affinity of ketamine to clinically relevant transporters. Mol Pharm 15:326–331

Kharasch ED, Labroo R (1992) Metabolism of ketamine stereoisomers by human liver microsomes. Anesthesiology 77:1201–1207

Kinoshita H, Tanaka N, Takakura A, Abe H, Kumihashi M, Shibayama T, Jamal M, Ito A, Tsutsui K, Kimura S, Iwase H, Ameno K (2017) An autopsy case of death by combined use of benzodiazepines and diphenidine. Soud Lek 62:40–43

Kjellgren A, Jonsson K (2013) Methoxetamine (MXE) – a phenomenological study of experiences induced by a "legal high" from the internet. J Psychoactive Drugs 45:276–286

Kohrs R, Durieux ME (1998) Ketamine: teaching an old drug new tricks. Anesth Analg 87:1186–1193

Koike H, Chaki S (2014) Requirement of AMPA receptor stimulation for the sustained antidepressant activity of ketamine and LY341495 during the forced swim test in rats. Behav Brain Res 271:111–115

Koinig H, Marhofer P, Krenn CG, Klimscha W, Wildling E, Erlacher W, Nikolic A, Turnheim K, Semsroth M (2000) Analgesic effects of caudal and intramuscular *S*(+)-ketamine in children. Anesthesiology 93:976–980

Kokkinou M, Ashok AH, Howes OD (2018) The effects of ketamine on dopaminergic function: meta-analysis and review of the implications for neuropsychiatric disorders. Mol Psychiatry 23:59–69

Kreuscher H (ed) (1969) Ketamine. Bericht über das internationale Symposion am 23. und 24. Februar 1968 in Mainz. Springer, Berlin

Krupitsky EM, Burakov AM, Romanova TN, Grinenko NI, Grinenko AY, Fletcher J, Petrakis IL, Krystal JH (2001) Attenuation of ketamine effects by nimodipine pretreatment in recovering ethanol dependent men: psychopharmacologic implications of the interaction of NMDA and L-type calcium channel antagonists. Neuropsychopharmacology 25:936–947

Krupitsky EM, Burakov AM, Dunaevsky IV, Romanova TN, Slavina TY, Grinenko AY (2007) Single versus repeated sessions of ketamine-assisted psychotherapy for people with heroin dependence. J Psychoactive Drugs 39:13–19

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB Jr, Charney DS (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 51:199–214

Kudo K, Usumoto Y, Kikura-Hanajiri R, Sameshima N, Tsuji A, Ikeda N (2015) A fatal case of poisoning related to new cathinone designer drugs, 4-methoxy PV8, PV9, and 4-methoxy PV9, and a dissociative agent, diphenidine. Leg Med (Tokyo) 17:421–426

Kusano M, Zaitsu K, Taki K, Hisatsune K, Nakajima J, Moriyasu T, Asano T, Hayashi Y, Tsuchihashi H, Ishii A (2017) Fatal intoxication by 5F-ADB and diphenidine: detection, quantification, and investigation of their main metabolic pathways in humans by LC/MS/MS and LC/Q-TOFMS. Drug Test Anal. https://doi.org/10.1002/dta.2215

Laher I, Zhang X, Leung PC, Liang W (2015) Diverse pharmacological properties of ketamine. In: Yew DT (ed) Ketamine. Use and abuse. CRC Press, Boca Raton, pp 37–63

Lahti AC, Koffel B, LaPorte D, Tamminga CA (1995) Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 13:9–19

Lalonde BR, Wallage HR (2004) Postmortem blood ketamine distribution in two fatalities. J Anal Toxicol 28:71–74

Lam RP, Yip WL, Tsui MS, Ng SW, Ching CK, Mak TW (2016) Severe rhabdomyolysis and acute kidney injury associated with methoxphenidine. Clin Toxicol (Phila) 54:464–465

Lawn W, Borschmann R, Cottrell A, Winstock A (2016) Methoxetamine: prevalence of use in the USA and UK and associated urinary problems. J Subst Use 21:115–120

Le Gall E, Troupel M, Nedelec JY (2006) One-step three-component coupling of aromatic organozinc reagents, secondary amines, and aromatic aldehydes into functionalized diarylmethylamines. Tetrahedron 62:9953–9965

Leander JD, Rathbun RC, Zimmerman DM (1988) Anticonvulsant effects of phencyclidine-like drugs: relation to N-methyl-D-aspartic acid antagonism. Brain Res 454:368–372

Li Q, Man Chan W, Rudd JA, Mei Wang C, Lam PYH, Mun Wai MS, Wood DM, Dargan PI, Yew DT (2013a) Ketamine. In: Dargan PI, Wood DM (eds) Novel psychoactive substances. Academic Press, Boston, pp 285–316

Li Y, Coller JK, Hutchinson MR, Klein K, Zanger UM, Stanley NJ, Abell AD, Somogyi AA (2013b) The CYP2B6*6 allele significantly alters the N-demethylation of ketamine enantiomers in vitro. Drug Metab Dispos 41:1264–1272

Li Y, Jackson KA, Slon B, Hardy JR, Franco M, William L, Poon P, Coller JK, Hutchinson MR, Currow DC, Somogyi AA (2015) CYP2B6*6 allele and age substantially reduce steady-state ketamine clearance in chronic pain patients: impact on adverse effects. Br J Clin Pharmacol 80:276–284

Licata M, Pierini G, Popoli G (1994) A fatal ketamine poisoning. J Forensic Sci 39:1314–1320

Lilly JC (1996) The scientist. A metaphysical autobiography, 3rd edn. Ronin Publishing, Oakland

Lim DK (2003) Ketamine associated psychedelic effects and dependence. Singap Med J 44:31–34

Lin F, He Y, Zhang L, Zhang M, Zhang Y, Wen C (2015) Assessment of the effect of ketamine on cytochrome P450 isoforms activity in rats by cocktail method. Int J Clin Exp Med 8:4335–4341

Livingston A, Waterman AE (1978) The development of tolerance to ketamine in rats and the significance of hepatic metabolism. Br J Pharmacol 64:63–69

Lodge D, Mercier MS (2015) Ketamine and phencyclidine: the good, the bad and the unexpected. Br J Pharmacol 172:4254–4276

Luethi D, Hoener MC, Liechti ME (2018) Effects of the new psychoactive substances diclofensine, diphenidine, and methoxphenidine on monoaminergic systems. Eur J Pharmacol 819:242–247

MacDonald JF, Miljkovic Z, Pennefather P (1987) Use-dependent block of excitatory amino acid currents in cultured neurons by ketamine. J Neurophysiol 58:251–266

Machado-Vieira R, Henter ID, Zarate CA Jr (2017) New targets for rapid antidepressant action. Prog Neurobiol 152:21–37

MacLennan FM (1982) Ketamine tolerance and hallucinations in children. Anaesthesia 37:1214–1215

Maixner J, Jurásek B, Kohout M, Kuchař M, Kačer P (2017) X-ray powder diffraction data for (S)-deschloroketamine hydrochloride, C13H18ClNO. Powder Diffract 32:193–195

Malhotra AK, Pinals DA, Weingartner H, Sirocco K, Missar CD, Pickar D, Breier A (1996) NMDA receptor function and human cognition: the effects of ketamine in healthy volunteers. Neuropsychopharmacology 14:301–307

Marcsekova K, Wegener B, Doye S (2005) Ind2TiMe2-catalyzed addition of methyl- and ethylamine to alkynes. Eur J Org Chem 4843–4851

Marietta MP, White PF, Pudwill CR, Way WL, Trevor AJ (1976) Biodisposition of ketamine in the rat: self-induction of metabolism. J Pharmacol Exp Ther 196:536–544

Maskell KF, Bailey ML, Rose SR (2016) Self medication with methoxetamine as an analgesic resulting in significant toxicity. Pain Med 17:1773–1775

Massmann V, Edemir B, Schlatter E, Al-Monajjed R, Harrach S, Klassen P, Holle SK, Sindic A, Dobrivojevic M, Pavenstädt H, Ciarimboli G (2014) The organic cation transporter 3 (OCT3) as molecular target of psychotropic drugs: transport characteristics and acute regulation of cloned murine OCT3. Pflugers Arch - Eur J Physiol 466:517–527

McCarthy DA (1981) History of the development of cataleptoid anesthetics of the phencyclidine type. In: Domino EF (ed) PCP (phencyclidine): historical and current perspectives. NPP Books, Ann Arbor, pp 17–24

McCarthy DA, Chen G, Kaump DH, Ensor C (1965) General anesthetic and other pharmacological properties of 2-(O-chlorophenyl)-2-methylamino cyclohexanone HCl (CI-581). J Clin Pharmacol 5:21–33

McLaughlin G, Morris N, Kavanagh PV, Power JD, O'Brien J, Talbot B, Elliott SP, Wallach J, Hoang K, Morris H, Brandt SD (2016) Test purchase, synthesis, and characterization of 2-methoxydiphenidine (MXP) and differentiation from its meta- and para-substituted isomers. Drug Test Anal 8:98–109

Mealing GA, Lanthorn TH, Small DL, Murray RJ, Mattes KC, Comas TM, Morley P (2001) Structural modifications to an N-methyl-D-aspartate receptor antagonist result in large differences in trapping block. J Pharmacol Exp Ther 297:906–914

Menzies EL, Hudson SC, Dargan PI, Parkin MC, Wood DM, Kicman AT (2014) Characterizing metabolites and potential metabolic pathways for the novel psychoactive substance methoxetamine. Drug Test Anal 6:506–515

Meyer MR, Bach M, Welter J, Bovens M, Turcant A, Maurer HH (2013a) Ketamine-derived designer drug methoxetamine: metabolism including isoenzyme kinetics and toxicological detectability using GC-MS and LC-(HR-)MS^n. Anal Bioanal Chem 405:6307–6321

Meyer MR, Orschiedt T, Maurer HH (2013b) Michaelis-Menten kinetic analysis of drugs of abuse to estimate their affinity to human P-glycoprotein. Toxicol Lett 217:137–142

Minakata K, Yamagishi I, Nozawa H, Hasegawa K, Wurita A, Gonmori K, Suzuki M, Watanabe K, Suzuki O (2015) Diphenidine and its metabolites in blood and urine analyzed by MALDI-Q-TOF mass spectrometry. Forensic Toxicol 33:402–408

Mion G, Villevieille T (2013) Ketamine pharmacology: an update (pharmacodynamics and molecular aspects, recent findings). CNS Neurosci Ther 19:370–380

Moaddel R, Venkata SL, Tanga MJ, Bupp JE, Green CE, Iyer L, Furimsky A, Goldberg ME, Torjman MC, Wainer IW (2010) A parallel chiral-achiral liquid chromatographic method for the determination of the stereoisomers of ketamine and ketamine metabolites in the plasma and urine of patients with complex regional pain syndrome. Talanta 82:1892–1904

Moghimi A, Rahmani S, Zare R, Sadeghzadeh M (2014) Synthesis of 2-(2-fluorophenyl)-2-methylamino-cyclohexanone as a new ketamine derivative. Synth Commun 44:2021–2028

Moore KA, Kilbane EM, Jones R, Kunsman GW, Levine B, Smith M (1997) Tissue distribution of ketamine in a mixed drug fatality. J Forensic Sci 42:1183–1185

Moreton JE, Meisch RA, Stark L, Thompson T (1977) Ketamine self-administration by the rhesus monkey. J Pharmacol Exp Ther 203:303–309
Morgan CJ, Curran HV (2012) Ketamine use: a review. Addiction (Abingdon, England) 107:27–38
Morris H (2011) Interview with a ketamine chemist or to be more precise, an arylcyclohexylamine chemist. Vice Mag 18:98–101
Morris H, Wallach J (2014) From PCP to MXE: a comprehensive review of the non-medical use of dissociative drugs. Drug Test Anal 6:614–632
Mutti A, Aroni S, Fadda P, Padovani L, Mancini L, Collu R, Muntoni AL, Fattore L, Chiamulera C (2016) The ketamine-like compound methoxetamine substitutes for ketamine in the self-administration paradigm and enhances mesolimbic dopaminergic transmission. Psychopharmacology 233:2241–2251
National Slovenian Forensic Laboratory (2016) Analytical report. Fluorolintane (C18H20FN). 1-(1-(2-fluorophenyl)-2-phenylethyl)pyrrolidine. National Slovenian Forensic Laboratory. https://www.policija.si/apps/nfl_response_web/0_Analytical_Reports_final/FLUOROLINTANE-ID-1420-15-report_final.pdf. Accessed 3 Feb 2018
Newcomer JW, Farber NB, Jevtovic-Todorovic V, Selke G, Melson AK, Hershey T, Craft S, Olney JW (1999) Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. Neuropsychopharmacology 20:106–118
Ng SH, Tse ML, Ng HW, Lau FL (2010) Emergency department presentation of ketamine abusers in Hong Kong: a review of 233 cases. Hong Kong Med J 16:6–11
Niciu MJ, Henter ID, Luckenbaugh DA, Zarate CA Jr, Charney DS (2014) Glutamate receptor antagonists as fast-acting therapeutic alternatives for the treatment of depression: ketamine and other compounds. Annu Rev Pharmacol Toxicol 54:119–139
Nishimura M, Sato K (1999) Ketamine stereoselectively inhibits rat dopamine transporter. Neurosci Lett 274:131–134
Nishimura M, Sato K, Okada T, Yoshiya I, Schloss P, Shimada S, Tohyama M (1998) Ketamine inhibits monoamine transporters expressed in human embryonic kidney 293 cells. Anesthesiology 88:768–774
Novelli A, Huidobro H (1963) Analogues <<ouverts>> de la papavérine. Activité spasmolytique type papavérine des dériveé de la 1,2(bis-diphényl)-éthylamine. Ann Pharm Fr 21:821–827
Oye I, Hustveit O, Maurset A, Ratti Moberg E, Paulsen O, Skoglund LA (1991) The chiral forms of ketamine as probes for NMDA receptor functions in humans. In: Kameyama T, Nabeshima T, Domino EF (eds) NMDA receptor related agents: biochemistry, pharmacology and behavior. NPP Books, Ann Arbor, pp 381–389
Oye I, Paulsen O, Maurset A (1992) Effects of ketamine on sensory perception: evidence for a role of N-methyl-D-aspartate receptors. J Pharmacol Exp Ther 260:1209–1213
Pal HR, Berry N, Kumar R, Ray R (2002) Ketamine dependence. Anaesth Intensive Care 30:382–384
Palmer GC, Hutchison JB (1997) Preclinical and clinical aspects of remacemide hydrochloride. In: Herrling PL (ed) Excitatory amino acids. Clinical results with antagonists. Academic Press, San Diego, pp 109–120
Parsons CG, Quack G, Bresink I, Baran L, Przegalinski E, Kostowski W, Krzascik P, Hartmann S, Danysz W (1995) Comparison of the potency, kinetics and voltage-dependency of a series of uncompetitive NMDA receptor antagonists in vitro with anticonvulsive and motor impairment activity in vivo. Neuropharmacology 34:1239–1258
Pomarol-Clotet E, Honey GD, Murray GK, Corlett PR, Absalom AR, Lee M, McKenna PJ, Bullmore ET, Fletcher PC (2006) Psychological effects of ketamine in healthy volunteers: phenomenological study. Br J Psychiatry 189:173–179
Preiss D, Tatar A (1995) Verwendung von 2-Methylamino-2-phenylcyclohexanon zur Behandlung von Bakterien-, Pilz-, Virus-, oder Protozoeninfektionen und zur Immunomodulation. Patent No. DE4409671. Berlin

Quirion R, Hammer RP Jr, Herkenham M, Pert CB (1981) Phencyclidine (angel dust)/sigma "opiate" receptor: visualization by tritium-sensitive film. Proc Natl Acad Sci U S A 78:5881–5885

Rao LK, Flaker AM, Friedel CC, Kharasch ED (2016) Role of cytochrome P4502B6 polymorphisms in ketamine metabolism and clearance. Anesthesiology 125:1103–1112

Ribeiro PO, Tome AR, Silva HB, Cunha RA, Antunes LM (2014) Clinically relevant concentrations of ketamine mainly affect long-term potentiation rather than basal excitatory synaptic transmission and do not change paired-pulse facilitation in mouse hippocampal slices. Brain Res 1560:10–17

Roth BL, Gibbons S, Arunotayanun W, Huang XP, Setola V, Treble R, Iversen L (2013) The ketamine analogue methoxetamine and 3- and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor. PLoS One 8:e59334

Roth BL, Gibbons S, Arunotayanun W, Huang XP, Setola V, Treble R, Iversen L (2018) Correction: the ketamine analogue methoxetamine and 3- and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor. PLoS One 13: e0194984

Salat K, Siwek A, Starowicz G, Librowski T, Nowak G, Drabik U, Gajdosz R, Popik P (2015) Antidepressant-like effects of ketamine, norketamine and dehydronorketamine in forced swim test: role of activity at NMDA receptor. Neuropharmacology 99:301–307

Salomone A, Gazzilli G, Di Corcia D, Gerace E, Vincenti M (2016) Determination of cathinones and other stimulant, psychedelic, and dissociative designer drugs in real hair samples. Anal Bioanal Chem 408:2035–2042

Sanacora G, Smith MA, Pathak S, Su HL, Boeijinga PH, McCarthy DJ, Quirk MC (2014) Lanicemine: a low-trapping NMDA channel blocker produces sustained antidepressant efficacy with minimal psychotomimetic adverse effects. Mol Psychiatry 19:978–985

Seeman P, Ko F, Tallerico T (2005) Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics. Mol Psychiatry 10:877–883

Sein Anand J, Wiergowski M, Barwina M, Kaletha K (2012) Accidental intoxication with high dose of methoxetamine (MXE) – a case report. Przegl Lek 69:609–610

Shahani R, Stewart RJ (2008) Reply to letter-to-the-editor, Re: Shahani R, Streutker C, Dickson B, et al: Ketamine-associated ulcerative cystitis: a new clinical entity. Urology 69: 810-812, 2007. 71:987

Shahani R, Streutker C, Dickson B, Stewart RJ (2007) Ketamine-associated ulcerative cystitis: a new clinical entity. Urology 69:810–812

Shields JE, Dargan PI, Wood DM, Puchnarewicz M, Davies S, Waring WS (2012) Methoxetamine associated reversible cerebellar toxicity: three cases with analytical confirmation. Clin Toxicol (Phila) 50:438–440

Simmler LD, Buchy D, Chaboz S, Hoener MC, Liechti ME (2016) In vitro characterization of psychoactive substances at rat, mouse, and human trace amine-associated receptor 1. J Pharmacol Exp Ther 357:134–144

Slovenian National Forensic Laboratory (2016) Analytical report. Deschloro-N-ethyl-ketamine (C14H19NO). Slovenian National Forensic Laboratory. https://www.policija.si/apps/nfl_response_web/0_Analytical_Reports_final/deschloro-N-ethyl-ketamine-ID-1607-16-rpt060816.pdf. Accessed 3 Feb 2018

Smith DJ, Azzaro AJ, Zaldivar SB, Palmer S, Lee HS (1981) Properties of the optical isomers and metabolites of ketamine on the high affinity transport and catabolism of monoamines. Neuropharmacology 20:391–396

Stevens CL (1962) Aminoketones and methods for their production. Patent no. US3254124A. Parke Davis, Detroit

Stevens CL (1968) α-Hydroxyimines and aminoketones. Patent No. US3394182A. Parke Davis, Detroit

Stevens CL, Elliott RD, Winch BL, Klundt IL (1962) A new rearrangement of α-aminoketones. J Am Chem Soc 84:2272–2274

Stevens CL, Elliott RD, Winch BL (1963) Aminoketone rearrangements. II. The rearrangement of phenyl α-aminoketones. J Am Chem Soc 85:1464–1470

Stevens CL, Klundt IL, Munk ME, Pillai MD (1965a) Amino ketone rearrangements. IV. Thermal rearrangements of α-amino methyl ketones. J Org Chem 30:2967–2972

Stevens CL, Thuillier A, Daniher FA (1965b) Amino ketone rearrangements. III. The rearrangement of α-hydroxy N-phenylimines. J Org Chem 30:2962–2966

Stevens CL, Ash AB, Thuillier A, Amin JH, Balys A, Dennis WE, Dickerson JP, Glinski RP, Hanson HT, Pillai MD, Stoddard JW (1966a) Amino ketone rearrangements. VI. Synthesis of 2-alkylamino-2-phenylcyclohexanones. J Org Chem 31:2593–2601

Stevens CL, Hanson HT, Taylor KG (1966b) Amino ketone rearrangements. V. A kinetic analysis. J Am Chem Soc 88:2769–2774

Stevens CL, Thuillier A, Taylor KG, Daniher FA, Dickerson JP, Hanson HT, Nielsen NA, Tikotkar NA, Weier RM (1966c) Amino ketone rearrangements. VII. Synthesis of 2-methylamino-2-substituted phenylcyclohexanones. J Org Chem 31:2601–2607

Stevens CL, Cahoon JM, Potts TR, Pillai PM (1972) Epoxyamines. III. Synthesis and reactions of 2-(1-aziridinyl)-2-phenyl-3,3-dimethyloxirane and 2-(1-aziridinyl)-2-phenyl-1-oxaspiro[2.4] heptane. J Org Chem 37:3130–3133

Stewart AT Jr, Hauser CR (1955) Synthesis and reactions of α-dialkylaminobenzyl butyl ethers. Interactions with Grignard reagents to form tertiary amines. J Am Chem Soc 77:1098–1103

Storr TM, Quibell R (2009) Can ketamine prescribed for pain cause damage to the urinary tract? Palliat Med 23:670–672

Striebel JM, Nelson EE, Kalapatapu RK (2017) "Being with a Buddha": a case report of methoxetamine use in a United States veteran with PTSD. Case Rep Psychiatry 2017:2319094

Suzuki K, Nosyreva E, Hunt KW, Kavalali ET, Monteggia LM (2017) Effects of a ketamine metabolite on synaptic NMDAR function. Nature 546:E1–E3

Svenningsson P, Nomikos GG, Greengard P (2004) Response to comment on "Diverse psychotomimetics act through a common signaling pathway". Science 305:180

Tainter ML, Luduena FP, Lackey RW, Neuru EN (1943) Actions of a series of diphenylethylamines. J Pharmacol Exp Ther 77:317–323

Tam SW, Zhang AZ (1988) σ and PCP receptors in human frontal cortex membranes. Eur J Pharmacol 154:343–344

Tan S, Chan WM, Wai MS, Hui LK, Hui VW, James AE, Yeung LY, Yew DT (2011) Ketamine effects on the urogenital system – changes in the urinary bladder and sperm motility. Microsc Res Tech 74:1192–1198

Tang HC, Lam PYH, Liang W (2015) In: Yew DT (ed) Ketamine. Use and abuse. CRC Press, Boca Raton, pp 227–242

Taschwer M, Hofer MG, Schmid MG (2014) Enantioseparation of benzofurys and other novel psychoactive compounds by CE and sulfobutylether β-cyclodextrin as chiral selector added to the BGE. Electrophoresis 35:2793–2799

Thurkauf A, Monn J, Mattson MV, Jacobson AE, Rice KC (1989) Structural and conformational aspects of the binding of aryl-alkyl amines to the phencyclidine binding site. In: Problems of drug dependence, 1989: Proceedings of the 51st annual scientific meeting, the Committee on Problems of Drug Dependence, Inc. NIDA Research Monograph, vol 95. United States Department of Health and Human Services, National Institute on Drug Abuse, Rockville, pp 51–56

Tsai TH, Cha TL, Lin CM, Tsao CW, Tang SH, Chuang FP, Wu ST, Sun GH, Yu DS, Chang SY (2009) Ketamine-associated bladder dysfunction. Int J Urol 16:826–829

Tso MM, Blatchford KL, Callado LF, McLaughlin DP, Stamford JA (2004) Stereoselective effects of ketamine on dopamine, serotonin and noradrenaline release and uptake in rat brain slices. Neurochem Int 44:1–7

Turfus SC, Parkin MC, Cowan DA, Halket JM, Smith NW, Braithwaite RA, Elliot SP, Steventon GB, Kicman AT (2009) Use of human microsomes and deuterated substrates: an alternative approach for the identification of novel metabolites of ketamine by mass spectrometry. Drug Metab Dispos 37:1769–1778

UK S.I. No. 239 (2013) The Misuse of Drugs Act 1971 (Amendment) Order 2013 (Statutory Instrument No. 239). http://www.legislation.gov.uk/uksi/2013/239/pdfs/uksi_20130239_en.pdf. Accessed 27 Mar 2018

Valli A, Lonati D, Locatelli CA, Buscaglia E, Tuccio MD, Papa P (2017) Analytically diagnosed intoxication by 2-methoxphenidine and flubromazepam mimicking an ischemic cerebral disease. Clin Toxicol (Phila) 55:611–612

Van Hout MC, Hearne E (2015) "Word of mouse": indigenous harm reduction and online consumerism of the synthetic compound methoxphenidine. J Psychoactive Drugs 47:30–41

Van Hout MC, Hearne E (2017) New psychoactive substances (NPS) on cryptomarket fora: an exploratory study of characteristics of forum activity between NPS buyers and vendors. Int J Drug Policy 40:102–110

Viceland (2017) Hamilton's pharmacopeia. Season 2. Ketamine; realms and realities. Viceland TV programme. Aired 26 Dec 2017

Vollenweider FX, Leenders KL, Øye I, Hell D, Angst J (1997) Differential psychopathology and patterns of cerebral glucose utilisation produced by (*S*)- and (*R*)-ketamine in healthy volunteers using positron emission tomography (PET). Eur Neuropsychopharmacol 7:25–38

Vutskits L (2018) General anesthetics to treat major depressive disorder: clinical relevance and underlying mechanisms. Anesth Analg 126:208–216

Wallach JV (2014) Structure activity relationship (SAR) studies of arylcyclohexylamines as N-methyl-D-aspartate receptor antagonists. Ph.D. dissertation, University of the Sciences, Philadelphia

Wallach J, Brandt SD (2018) Phencyclidine-based new psychoactive substances. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_124

Wallach J, Kavanagh PV, McLaughlin G, Morris N, Power JD, Elliott SP, Mercier MS, Lodge D, Morris H, Dempster NM, Brandt SD (2015) Preparation and characterization of the 'research chemical' diphenidine, its pyrrolidine analogue, and their 2,2-diphenylethyl isomers. Drug Test Anal 7:358–367

Wallach J, Kang H, Colestock T, Morris H, Bortolotto ZA, Collingridge GL, Lodge D, Halberstadt AL, Brandt SD, Adejare A (2016) Pharmacological investigations of the dissociative 'legal highs' diphenidine, methoxphenidine and analogues. PLoS One 11:e0157021

Wang S, Li C (1987) Synthesis of anesthetic compound 2-(O-fluorophenyl)-2-methylamino-cyclohexanone hydrochloride (F-ketamine). Acta Sci Nat Univ Pekin 116–119

Wang Q, Wu Q, Wang J, Chen Y, Zhang G, Chen J, Zhao J, Wu P (2017) Ketamine analog methoxetamine induced inflammation and dysfunction of bladder in rats. Int J Mol Sci 18:E117

Ward J, Rhyee S, Plansky J, Boyer E (2011) Methoxetamine: a novel ketamine analog and growing health-care concern. Clin Toxicol (Phila) 49:874–875

Watterson J (2015) Postmortem toxicology of ketamine. In: Yew DT (ed) Ketamine. Use and abuse. CRC Press, Boca Raton, pp 243–248

Weiner AL, Vieira L, McKay CA, Bayer MJ (2000) Ketamine abusers presenting to the emergency department: a case series. J Emerg Med 18:447–451

Weiß JA, Mohr S, Schmid MG (2015) Indirect chiral separation of new recreational drugs by gas chromatography-mass spectrometry using trifluoroacetyl-L-prolyl chloride as chiral derivatization reagent. Chirality 27:211–215

Westphal F, Junge T, Jacobsen-Bauer A, Rösner P (2010) Lefetamin-Derivate: alte Bekannte neu auf dem Drogenmarkt. Toxichem Krimtech 77:46–58

WHO (2014) Methoxetamine. Critical review report. Agenda item 4.22. In: Expert Committee on Drug Dependence thirty-sixth meeting, World Health Organization, Geneva, 16–20 June 2014. http://www.who.int/medicines/areas/quality_safety/4_22_review.pdf. Accessed 3 Feb 2018

WHO (2015) MT-45. Critical review report agenda item 5.1. In: Expert Committee on Drug Dependence thirty-seventh meeting, World Health Organization, Geneva, 16–20 Nov 2015. http://www.who.int/medicines/access/controlled-substances/5.1_MT-45_CRev.pdf. Accessed 27 Mar 2018

Wieber J, Gugler R, Hengstmann JH, Dengler HJ (1975) Pharmacokinetics of ketamine in man. Anaesthesist 24:260–263

Wikstrom M, Thelander G, Dahlgren M, Kronstrand R (2013) An accidental fatal intoxication with methoxetamine. J Anal Toxicol 37:43–46

Wink CS, Meyer GM, Wissenbach DK, Jacobsen-Bauer A, Meyer MR, Maurer HH (2014) Lefetamine-derived designer drugs N-ethyl-1,2-diphenylethylamine (NEDPA) and N-iso-propyl-1,2-diphenylethylamine (NPDPA): metabolism and detectability in rat urine using GC-MS, LC-MS and LC-HR-MS/MS. Drug Test Anal 6:1038–1048

Wink CSD, Meyer GMJ, Meyer MR, Maurer HH (2015) Toxicokinetics of lefetamine and derived diphenylethylamine designer drugs-contribution of human cytochrome P450 isozymes to their main phase I metabolic steps. Toxicol Lett 238:39–44

Wink CS, Michely JA, Jacobsen-Bauer A, Zapp J, Maurer HH (2016) Diphenidine, a new psychoactive substance: metabolic fate elucidated with rat urine and human liver preparations and detectability in urine using GC-MS, LC-MS(n), and LC-HR-MS(n). Drug Test Anal 8:1005–1014

Winstock AR, Lawn W, Deluca P, Borschmann R (2016) Methoxetamine: an early report on the motivations for use, effect profile and prevalence of use in a UK clubbing sample. Drug Alcohol Rev 35:212–217

Wood DM, Davies S, Puchnarewicz M, Johnston A, Dargan PI (2012) Acute toxicity associated with the recreational use of the ketamine derivative methoxetamine. Eur J Clin Pharmacol 68:853–856

Xie L-G, Dixon DJ (2017) Tertiary amine synthesis via reductive coupling of amides with Grignard reagents. Chem Sci 8:7492–7497

Yanagihara Y, Kariya S, Ohtani M, Uchino K, Aoyama T, Yamamura Y, Iga T (2001) Involvement of CYP2B6 in N-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 29:887–890

Yeung LY, Rudd JA, Lam WP, Mak YT, Yew DT (2009) Mice are prone to kidney pathology after prolonged ketamine addiction. Toxicol Lett 191:275–278

Yuizono T, Matsuo S, Nakama M, Kase Y, Fujimura H (1970) Chemico-pharmacological studies on antitussives. X. Pharmacological studies on 1,2-diphenyl-1-tert-aminoethane derivatives. Comparison of the optical isomers of 1,2-diphenyl-1-pyrrolidinoethane. Yakugaku Zasshi 90:24–31

Zanda MT, Fadda P, Chiamulera C, Fratta W, Fattore L (2016) Methoxetamine, a novel psychoactive substance with serious adverse pharmacological effects: a review of case reports and preclinical findings. Behav Pharmacol 27:489–496

Zanda MT, Fadda P, Antinori S, Di Chio M, Fratta W, Chiamulera C, Fattore L (2017) Methoxetamine affects brain processing involved in emotional response in rats. Br J Pharmacol 174:3333–3345

Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, Alkondon M, Yuan P, Pribut HJ, Singh NS, Dossou KS, Fang Y, Huang XP, Mayo CL, Wainer IW, Albuquerque EX, Thompson SM, Thomas CJ, Zarate CA Jr, Gould TD (2016) NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature 533:481–486

Zanos P, Thompson SM, Duman RS, Zarate CA Jr, Gould TD (2018) Convergent mechanisms underlying rapid antidepressant action. CNS Drugs. https://doi.org/10.1007/s40263-40018-40492-x

Zarate CA Jr, Brutsche N, Laje G, Luckenbaugh DA, Venkata SL, Ramamoorthy A, Moaddel R, Wainer IW (2012) Relationship of ketamine's plasma metabolites with response, diagnosis, and side effects in major depression. Biol Psychiatry 72:331–338

Zarate CA Jr, Mathews D, Ibrahim L, Chaves JF, Marquardt C, Ukoh I, Jolkovsky L, Brutsche NE, Smith MA, Luckenbaugh DA (2013) A randomized trial of a low-trapping nonselective N-methyl-D-aspartate channel blocker in major depression. Biol Psychiatry 74:257–264

Zawilska JB (2014) Methoxetamine – a novel recreational drug with potent hallucinogenic properties. Toxicol Lett 230:402–407

Zeilhofer HU, Swandulla D, Geisslinger G, Brune K (1992) Differential effects of ketamine enantiomers on NMDA receptor currents in cultured neurons. Eur J Pharmacol 213:155–158

Zhang DX, Levy WB (1992) Ketamine blocks the induction of LTP at the lateral entorhinal cortex-dentate gyrus synapses. Brain Res 593:124–127

Zhang JC, Li SX, Hashimoto K (2014) *R* (−)-ketamine shows greater potency and longer lasting antidepressant effects than *S* (+)-ketamine. Pharmacol Biochem Behav 116:137–141

Zwartsen A, Verboven AHA, van Kleef R, Wijnolts FMJ, Westerink RHS, Hondebrink L (2017) Measuring inhibition of monoamine reuptake transporters by new psychoactive substances (NPS) in real-time using a high-throughput, fluorescence-based assay. Toxicol In Vitro 45:60–71

Synthetic Opioids

Patrick M. Beardsley and Yan Zhang

Contents

Abstract

Opioid abuse has been a global menace for centuries, but the proliferation of synthetic opioids and their use within this current decade have created epidemic-level harms in some countries. According to the United Nations Office on Drugs and Crime, almost 12 million years were estimated loss of "healthy" life resulting

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in premature death and disability attributable to global opioid abuse just in 2015. Law enforcement and regulatory authorities have been particularly challenged abating the spread of synthetic opioids because soon after controlling the currently recognized abused opioids, their structures are tweaked, and new entities replace them. Drug racketeers have most often exploited the fentanyl phenylpiperidine structure in this regard, but non-fentanyl-like and classical morphinan-like structures have been pirated as well. A growing number of anecdotal reports identify respiratory depression induced by these newer synthetic opioids to be especially refractive to reversal by antagonists, with consequently high levels of lethality. This review examines three of these synthetic opioids representing three chemical classes (U-47700, MT-45, and acetylfentanyl) that have emerged to be of such menace that they have been brought under international control in recent years and addresses factors that could make synthetic opioids especially untreatable by opioid antagonists.

Keywords
Acetylfentanyl · MT-45 · NPS · Opioid · Synthetic opioid · U-47700

Acronyms of the Discussed New Psychoactive Substances (NPS)

AH-7921	3,4-Dichloro-*N*-{[1-(dimethylamino)cyclohexyl]methyl}benzamide
MT-45	1-Cyclohexyl-4-(1,2-diphenylethyl)piperazine
U-47700	3,4-Dichloro-*N*-[(1*R*,2*R*)-2-(dimethylamino)cyclohexyl]-*N*-methylbenzamide
U-50488	2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*R*,2*R*)-2-(pyrrolidin-1-yl)cyclohexyl]acetamide
U-51754	2-(3,4-Dichlorophenyl)-*N*-[(1*R*,2*R*)-2-(dimethylamino)cyclohexyl]-*N*-methylacetamide

1 Introduction

The global opioid crisis is staggering. Almost 12 million years were estimated loss of "healthy" life resulting in premature death and disability attributable to global opioid abuse just in 2015 (UNODC 2017a). This loss caused by opioid abuse was about 70% of the total global burden of disease caused by all drug abuse disorders (UNODC 2017a). Although a worldwide catastrophe, the harm caused by opioid use has been most apparent in the United States. In 2016, 42,249 deaths occurred in the United States that involved opioid use, which accounted for about 66% of all drug overdose deaths (Seth et al. 2018). This estimate was indicative of aggressive escalation considering the nearly fivefold increase in deaths that occurred relative to those in 1999 (CDC 2017).

The opioid-use trajectory in the United States is illustrative. The present opioid crisis in the United States was established in three waves (CDC 2017). The first wave began in the 1990s propelled by the increase in the prescribing of opioids that was followed by increases in prescription opioid overdose deaths. The second wave began in 2010 with an unprecedented increase in overdose deaths involving heroin. This was followed by the most recent wave that began in 2013 with precipitous increases in overdose deaths involving synthetic opioids (CDC 2017). The infusion of synthetic opioids has taken the crisis to new levels, not only in the United States, but it is beginning to do so globally as well (UNODC 2017b).

Synthetic opioids are comprised primarily of fentanyl-related drugs in addition to a few drugs neither sharing in a fentanyl-like nor the classical morphinan chemical structure. It is difficult to estimate the number of these drugs that might be available to users because near-countless modifications of the structure of fentanyl alone are possible. As an indication of this potential variety, East Asia, Europe, and North America reported 14 fentanyl analogs and 3 non-fentanyl-related synthetic opioids to the United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory on new psychoactive substances just between 2012 and 2016 (UNODC 2017b). These drugs are most commonly sold as adulterations in mixtures with heroin or as counterfeit pharmaceuticals resembling prescription pills such as hydrocodone or oxycodone (UNODC 2017b; CDC 2018; Sutter et al. 2017). Many routes of administration have been known to be used with the synthetic opioids including oral, insufflation, intravenous, rectal, via an inhaler using a liquid solution, smoking, and via a vaporizer (e.g., WHO 2015a, b; 2016). Their potency ranges greatly from those about equipotent to that of morphine such as is valerylfentanyl (i.e., NIH 10488) (Aceto et al. 1988) to those $>10{,}000\times$ more potent such as is carfentanil (Vuckovic et al. 2009; Van Bever et al. 1976).

Several reviews of synthetic opioids have been published in recent years. Searching (20180412) on "(synthetic opioid OR fentanyl) AND abuse AND review (as document type) AND 2015-2018" in the Web of Science yielded 25 reviews. Scanning through these reviews, 7 were not pertinent leaving 18 on this subject just published in the last ~3 years. This review takes a different approach to most of these previous reviews and provides detailed examinations of three synthetic opioids chosen because they represent examples from three different chemical classes of compounds and because they individually have come forward into such prominence within the last 3 years that they have been brought under international control within the United Nations Single Convention on Narcotic Drugs. The three drugs to be described are U-47700, a benzamide; MT-45, a piperazine; and acetylfentanyl, a phenylpiperidine. Because there has been an emergence of reports that the respiratory depressant effects often leading to lethality induced by synthetic opioids have been uniquely refractory to reversal by naloxone (the most often used clinically approved reviving antagonist), mechanisms by which this may or not be true are discussed. The review concludes with a snapshot of the current harms of synthetic opioids and recent international legislation to control them.

2 U-47700

2.1 Chemistry and Detection

U-47700 is an analog of compound AH-7921 (Fig. 1) (Brittain et al. 1973). AH-7921 was initially developed by the Allen & Hanburys pharmaceutical company (Harper and Veitch 1976). It works as a μ-opioid receptor (MOR) agonist and can excite κ-opioid receptors (KOR) at high doses (see Sect. 2.2). Animal studies have shown that AH-7921 possesses typical morphine-like actions (Katselou et al. 2015), and because of the aggressive emergence of its global abuse, AH-7921 was included in the 1961 Single Convention on Narcotic Drugs in 2015 (Commission on Narcotic Drugs 2015). To further explore the structure-activity relationship of AH-7921, a number of its analogs were synthesized, and U-47700 was the most potent among them (Michalson and Szmuszkovicz 1989; Mullins 1966; Harper and Veitch 1976, 1977; Kalamazoo 1970; Szmuszkovicz 1970; Rynbrandt and Skaletzky 1972; Roll 1974; Collins et al. 1984; Casy and Parfitt 1986).

2.1.1 Synthesis of U-47700

U-47700 was synthesized by the Upjohn pharmaceutical company in the 1970s (Kalamazoo 1970; Szmuszkovicz 1970). The synthetic route of U-47700 is shown in Fig. 2. Its chemical synthesis is non-problematic and can be accomplished in non-sophisticated environments.

2.1.2 Forensic Detection

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is the principal method used to detect U-47700 in human urine and blood samples. U-47700 has been detected at a concentration of 224 ng/mL in urine using LC-MS/MS and

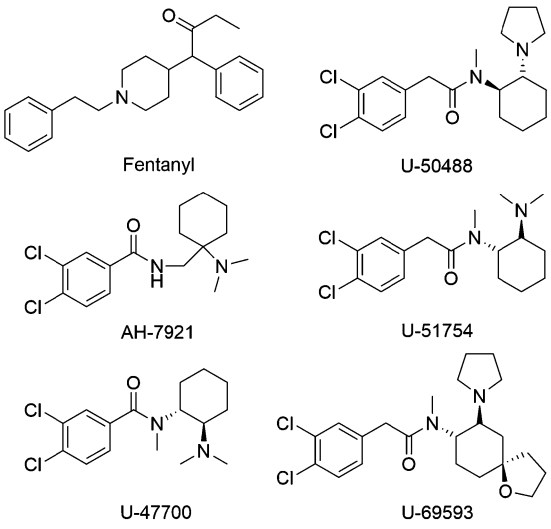

Fig. 1 Structures of U-47700 and its related analogs

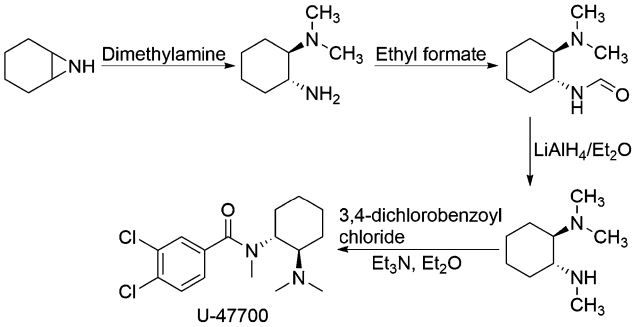

Fig. 2 The synthesis of U-47700

LC-QTOF (Fleming et al. 2017). The parent drug (U-47700) and related metabolites can also be detected by this method. In addition, U-47700 can also be identified in blood by similar methods (Mohr et al. 2016).

2.1.3 Analogs of U-47700

Though further development of U-47700 appears now abandoned, several selective KOR ligands had been synthesized based on the structure of U-47700, including U-50488, U-51754, and U-69593 (Fig. 1) (Zhao et al. 2000; Loew et al. 1988a; Szmuszkovicz 1999; Tsibulnikov et al. 2015). As a lead compound, several possible positions in the structure of U-47700 can be modified (Fig. 3), such as the a-ring or b-ring, the substituent groups (X, R, R_1, and R_2), or the related structure isomer. Much work has been conducted on the structural isomer of U-50488, U-50488H (Fig. 4), and a series of analogs of U-50488H have been synthesized and tested as selective KOR ligands (Barber and Gottschlich 1997).

2.2 Pharmacology

Lowe et al. evaluated U-47700 for its in vitro binding activity using guinea pig brain at the MOR and the KOR. U-47700 had high affinity at the MOR and bound with a K_D of 5.3 nM and weakly at the KOR with a K_D of 910 nM resulting in a >171× preference for the MOR relative to the KOR (Loew et al. 1988b). Subsequently these researchers reported U-47700 receptor affinities (IC_{50}s) of 9 and 300 nM, respectively, for the MOR and KORs (Loew et al. 1989).

Janowsky provided in vitro binding and in vitro functional activity results for U-47700 at μ, κ, and δ-opioid receptors (Janowsky 2016). Receptor binding studies were conducted using human δ-opioid receptor (DOR) and KORs transfected into Chinese hamster ovary (CHO) cells, and rat MORs transfected into CHO cells. In vitro functional activity was also evaluated by evaluating stimulation of [^{35}S]GTPγS binding activity using respective CHO cells at each of the opioid receptors. U-47700 potently bound to the MOR with a K_i (± SEM) of 0.91 (±0.11) nM, much less well

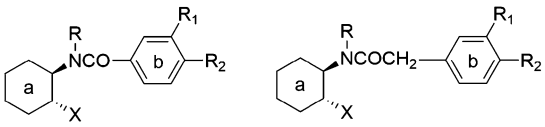

Fig. 3 The two general structures of U-47700 and its analogs

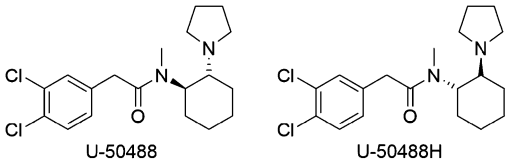

Fig. 4 The structure of U-50488 and U-50488H

to the KOR at 110 nM (±11), and poorly so to the DOR at 480 nM (±110). In comparison, morphine had K_i (±SEM) values at the MOR, KOR, and DORs of 0.213 (±0.019), 27.9 (±2.7), and 111 (±14) nM, respectively. Functional activity results at the MOR, KOR, and DORs in the [^{35}S]GTPγS assay [EC_{50} (±SEM)] for U-47700 were 140 (±23), 201 (±74), and 4,540 (±350) nM, respectively, and for morphine as a comparator, 31.0 (±8.2), 83 (±23), and 870 (±140) nM, respectively (Janowsky 2016).

In in vivo studies, Szmuszkovicz and Von Voigtlander reported potent antinociceptive activity in the mouse with U-47700 with ED_{50}s of 0.2, 0.2, and 0.2 mg/kg (sc) in the tail flick, tail pinch, and HCl writhing assays, respectively. U-47700 caused mice to fail the inclined screen test with an ED_{50} of 9.0 mg/kg suggesting some sedative-like effects. U-47700 also showed full overt morphine-like effects and induced Straub tail, arched back, and increased locomotion (Szmuszkovicz and Von Voigtlander 1982). Others have reported that U-47700 induces potent (7.5× > morphine) antinociception in the mouse tail flick assay with an ED_{50} of 0.2 mg/kg (sc) and produces characteristic rodent MOR agonist effects of Straub tail, arched back, and elevation of locomotor activity (Cheney et al. 1985).

There appears much opportunity for evaluating U-47700 in controlled, abuse liability-relevant studies because no controlled, laboratory animal or human studies regarding the reinforcing, discriminative stimulus, or dependence effects of U-47700 could be identified at the time of this review [searching on: "U-47770 AND (self-administration OR drug discrimination OR physical dependence)," 20180417].

2.3 Evidence of Abuse

U-47700 has been readily obtainable through the Internet often supplied via vendors advertised as providing research chemicals (DEA 2016a, b). Its availability may be diminishing with increasing national and international controls. Conducting a search using the Google search engine on the term, "U-47700 for sale," resulted in ~802,000 hits on 20161001 but only ~246,000 hits on 20180417. Some of these hits resulted in vendors obviously trying to sell products marketed as U-47700 [1) U-47700 buy U-47700 for sale online - US$39.20 -Best-Feel.com, 2) U-47700 buy U-47700 online

for sale - US$86.80 - BestRCS.com, 3) I just got some U-47700 - AMA : RCSources - Reddit, 4) Buy U-47700 online from Mr Chemistry. - Mrchemistry.com, 5) Buy Online U-47700 USA, UK, EU, AU @ US$8 per g: ChingLabs]. Other researchers have reported the ease of buying U-47700 online for as little as US$ 40 (Domanski et al. 2016). When purchased from the Internet, U-47700 is typically provided as a white powder, although it has been sold as a liquid as well (WHO 2016). Several routes of administration have been used as reported by users, including via insufflation, inhalation, intravenous, and rectal routes (e.g., Jesusgreen 2014; ElectronicExorcist 2016).

U-47700 use and/or seized material has been reported in several countries including Belgium, Denmark, Estonia, Finland, France, the United States, the United Kingdom, Scotland, Spain, and Sweden (e.g., Jones et al. 2016; Helander and Backberg 2016; Elliott et al. 2016; Coopman et al. 2016; EMCDDA 2016). Substantial seizures of U-47700 have occurred in the United States, in Spain, and in Scotland. For example, law enforcement officers in Lorain County, Ohio, USA, seized 500 pills in a single seizure of U-47700 during March 2016 that visually appeared to be oxycodone. The pills were blue and had "A 215" markings, consistent with 30-mg oxycodone pills. Laboratory analysis indicated that the pills did not contain oxycodone but were instead U-47700 (DEA 2016a). A single seizure of U-47700 in powder form was made on January 18, 2016 in Spain (Adolfo Suárez-Barajas Airport, Madrid), where 1.054 kg was seized in transit from China to Barcelona (EMCDDA 2016). And in Scotland, 2,626 tablets containing U-47700 were seized by police in three incidents between January and March 2016. The tablets featured three different designs of counterfeit diazepam (Police Scotland 2016a, b; EMCDDA 2016).

One of the earliest case reports of a patient presenting with an opioid-like toxidrome associated with U-47700 use was by Armenian and colleagues (Armenian et al. 2016). In their case report, a 41-year-old woman presented to an emergency department in Northern California after consuming three pills she thought were "Norco" pills, a street name referring to pills containing fentanyl and various amounts of acetaminophen and hydrocodone. She had regularly been purchasing acetaminophen-hydrocodone combination pills on the street for chronic back pain and took 2–3 at a time, 2–3 times a day. She presented with pinpoint eyes, respiratory depression, and depressed consciousness. She promptly awoke and was able to answer questions following intravenous administration of 0.4 mg of naloxone. During the subsequent 2 h, she remained somnolent but was able to wake up and speak coherently. Serum samples were analyzed, and results were significant for the presence of fentanyl (15 ng/mL) and U-47700 (7.6 ng/mL), along with several other drugs including acetaminophen, benzoylecgonine, gabapentin, hydrocodone, and sertraline (Armenian et al. 2016).

Another report of toxic use presumably of U-47700 was of a 22-year-old man with a history of heroin abuse who was found unconscious and apneic by his mother (Schneir et al. 2016). Paramedics administered 2 mg of naloxone intravenously that completely reversed his coma and bradypnea. The patient reported that just before being found by his mother, he had used U-47700 he had acquired over the Internet.

He described having purchased what he interpreted as 250 mg of the drug in powder form, which he divided into five separate doses. He had administered the drug by placing it in a syringe, mixing with water, and applying to his nostrils. Analysis of urine was performed using a broad-spectrum liquid chromatography time-of-flight (LC-TOF) high-resolution mass spectrometry assay that indicated urine was negative for 61 compounds but confirmed the presence of a compound with the molecular formula $C_{16}H_{22}C_{l2}N_2O$, which matched that of U-47700. Analysis ruled out the presence of AH-7921 that has the same molecular formula as U-47700 but a different structure. Ultimately, the patient had been resuscitated quickly with the aid of naloxone and appeared to not suffer permanent sequelae (Schneir et al. 2016). Other recent studies have reported adverse effects of U-47700 to be opioid-like following U-47700s acute administration (Domanski et al. 2016).

Elliott et al. (2016) reported one of the earliest fatalities associated with U-47700. A 27-year-old male was found dead in his home in January 2016. Routine toxicological analysis of the postmortem urine or blood detected quetiapine, amphetamine, amitriptyline, naproxen, mexedrone, and ketamine. Ethanol was not detected. Analysis confirmed the primary compound detected in powder taken from the decedent's nasal passage and his postmortem blood and urine to be U-47700. Quantitative analysis by HPLC-DAD of postmortem femoral blood measured U-47700 at a concentration of 1.46 mg/L. These researchers concluded, "The major risk to life from opioids is their depressant effect on the central nervous system, notably causing respiratory depression, and in the absence of any other significant pathological or toxicological findings, fatal U-47700 toxicity was a likely outcome" (Elliott et al. 2016).

Approximately at the same time as Elliott and colleagues reported their U-47700-associated death (Elliott et al. 2016), Coopman and colleagues had reported another (Coopman et al. 2016). In this instance, a 30-year-old man was found dead on the ground of a storage room in his house. Drug paraphernalia were present on the table in the decedent's living room including a recently delivered envelope from China containing a white powder (36 g). The powder was analyzed with GC-MS and found to contain trace amounts of fentanyl (0.0035%, m/m) and U-47700 (0.0012%, m/m). A toxic fentanyl level of 10.9 μg/L and a therapeutic level of 180 μg/L of sertraline were measured in the subclavian blood. Further analyses with UPLC–MS/MS revealed the presence of U-47700 at a concentration of 13.8 μg/L in blood and 71.0 μg/L in urine. The authors reported, "Based on circumstantial evidence (police investigation, crime scene) and the results of the toxicological analysis, the medical examiner concluded that the cause of death was an acute intoxication and overdose with fentanyl and U-47700 immediately after inhaling the fumes of the vaporized powder" (Coopman et al. 2016). However, Ruan et al. (2016) suggested that the degree to which U-47700 contributed to the decedent's death remained uncertain.

There have been several other reports of fatalities in which U-47700 have been associated. The Belgium Early Warning System on Drugs (BEWSD) of the Scientific Institute of Public Health reported in February 2016 that they received information on a fatality involving U-47700 and fentanyl (UNODC 2016). Postmortem blood samples, as well as powder seized, revealed the presence of fentanyl and U-47700.

The BEWSD advised that the substances were not sold as heroin (UNODC 2016). In separate incidents, one 20- and one 27-year-old man were found dead in their homes, and postmortem analysis of blood identified the presence of U-47700 in both cases. Analysis of powder discovered in the vicinity of the 27-year-old decedent indicated the presence of U-47700 and etizolam, although etizolam was not detected in blood (Spargo 2016). In 20 recent postmortem cases in which the deaths were initially believed to be associated with heroin or other opioid-related drug overdoses, U-47700 was the confirmed drug in 11 of the 20 cases, 5 cases of which were confirmed for both U-47700 and furanylfentanyl (Mohr et al. 2016). The Drug Enforcement Agency of the United States reported in September of 2016 that there had been at least 15 confirmed fatalities in the United States associated with U-47700 usage (DEA 2016b). However, it is uncertain whether the DEA was including the deaths reported in the Mohr et al. (2016) study cited above (DEA 2016b). Since these initial reports of U-47700-induced toxicity emerged in 2016, the number of fatal and near-fatal intoxications with U-47700 has briskly accelerated (Ellefsen et al. 2017; Harding et al. 2017; Jones et al. 2017; McIntyre et al. 2017; Nikolaou et al. 2017; O'Donnell et al. 2017; Prekupec et al. 2017; Schneir et al. 2016; Seither and Reidy 2017; Shoff et al. 2017).

As a consequence of its identified abuse and harms, U-4770 is controlled under law by several countries including the United States, the United Kingdom, Canada, and Sweden, and it was recently brought under international control in 2017 under the Single Convention on Narcotic Drugs, 1961 (INCB 2017).

3 MT-45

3.1 Chemistry and Detection

In the 1970s, analogs featuring the skeleton of 10,11-dihydrodibenzo[*b*,*f*]thiepine were reported to have central nervous system effects. Among them, perathiepin was then launched into clinical use as a tranquilizer (Fig. 5) (Patton et al. 2014). In order to obtain novel lead compounds with more favorable analgesic efficacy and fewer adverse effects, researchers postulated that ring opening, and removal of the sulfur atom might be desirable. Meanwhile, lefetamine (Fig. 5), a 1,2-diphenylethylamine and related to 1-(1,2-diphenylethyl)piperazine derivatives, was synthesized and demonstrated about one-tenth the potency of (−)-morphine, (Fujimura and Kawai 1960). Taken together, analogs bearing the piperazine moiety as well as the 1,2-diphenylethyl moiety were investigated as potential analgesics (Figs. 6 and 7).

Thus, a series of compounds with various substituents attached to one of the nitrogen atoms on the piperazine ring were synthesized (Fig. 6) (Natsuka et al. 1975). Among them, a compound with a cyclohexyl group (MT-45, Fig. 4) possessed outstanding analgesic activity. In addition, in structure-activity relationship (SAR) studies, it revealed that the 1,2-diphenylethyl moiety appeared to be necessary for retaining activity and a cycloalkyl group having 6–8 carbon atoms on piperazinyl moiety were thought to be more favorable for analgesic activity (Fig. 7)

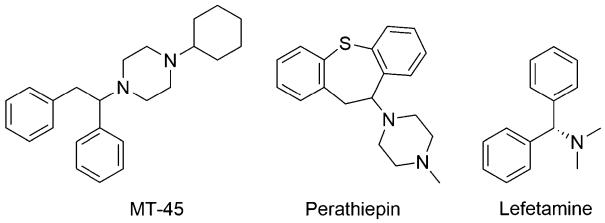

Fig. 5 Structures of MT-45, perathiepin, and lefetamine

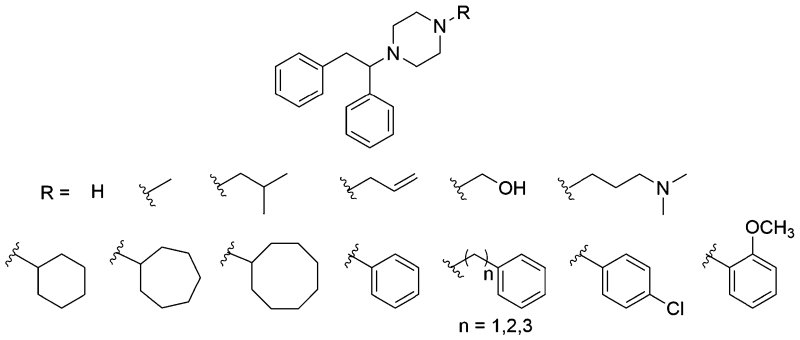

Fig. 6 Derivatives with substituents on piperazinyl moiety (Natsuka et al. 1975)

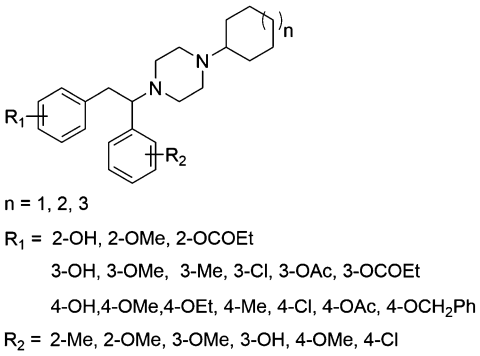

Fig. 7 Derivatives with substituents on 1-phenyl and benzyl groups (Natsuka et al. 1987)

(Natsuka et al. 1987). A follow-up study on the SAR of the two phenyl groups concluded that when meta-substituents were introduced on the benzyl group the potency order was OH $\geq$ OAc >> OMe $\geq$ H $\geq$ Me$\geq$Cl, whereas for para-substituted derivatives on the 2-phenyl group, the potency order was H $\geq$ OH (or OAc)> > OMe $\geq$ Me$\geq$Cl. On the other hand, the introduction of different

substituents on the phenyl group led to comparable decreases in analgesic activity (Natsuka et al. 1987).

Though it seems that the SAR of MT-45 has been exhausted, there still remains the possibility of modifying the cyclohexyl ring system based on access to varied substituted cyclohexylamines and convenient synthetic methods. Particularly, introduction of more hydrophobic moieties at this position, for example, alkyl substituted cyclohexyl ring systems, may lead to more potent analgesics and potential drugs of abuse.

3.1.1 Synthesis

Natsuka et al. (1975) originally reported the synthetic route of MT-45 (Fig. 8). Subsequently, a patent disclosed a new approach for synthesizing MT-45 that was superior to the former as it involved fewer steps and cheaper chemicals (Fig. 9) (Benchikh 2017). While both routes involve the preparation and handling of Grignard Reagents, MT-45 is not expected to be easily produced in a routine environment.

3.1.2 Forensic Detection

To date, several analytical methods have been utilized such as gas chromatography (GC) and LC-MS for analyzing the presence of MT-45 in biological material (Papsun et al. 2016). Recently, a US patent reported an immunological method to

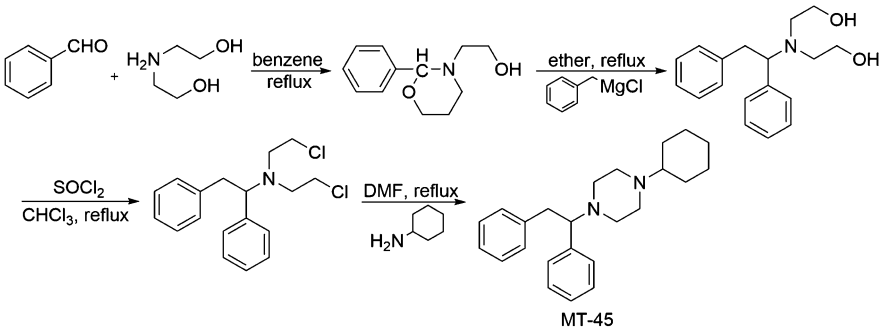

Fig. 8 Original synthetic route for MT-45

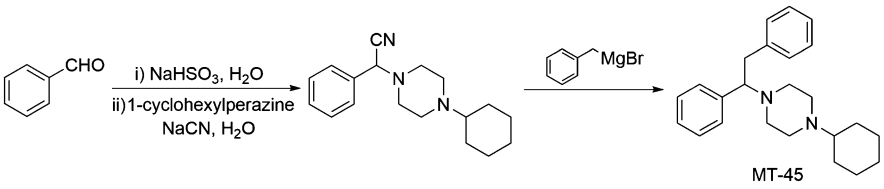

Fig. 9 Recently reported synthetic route for MT-45

identify MT-45 in a biological sample, which would facilitate MT-45 detection during routine screening (Benchikh 2017).

3.2 Pharmacology

Fujimura and colleagues characterized the inhibitory effect of MT-45 and its enantiomers on the stereospecific binding of 5×10^{-9} M [^{3}H]naloxone or 1×10^{-8} M [^{3}H]morphine to homogenates of rat brain (without cerebella) (Fujimura et al. 1978). The IC_{50} (μM) against naloxone binding was 0.74, 0.14, and 1.18 for the racemate, (*S*)-(+)- and (*R*)-(−)-isomers, respectively, all much less potent than morphine at 0.0055 (Fujimura et al. 1978). In other studies, Fujimura also evaluated MT-45 and its enantiomers for their binding affinities at MOR, KOR, and DOR using [^{3}H]dihydromorphine (70 Ci/mmol), [^{3}H]D-Ala^2-D-Leu^5-enkephalin (DADLE, 27.4 Ci/mmol), and [^{3}H]ethylketocyclazocine (EKC, 21.8 Ci/mmol), respectively (Nozaki et al. 1983). Morphine bound to the μ, δ, and κ opioid receptor sites with IC_{50}s (nM) of 4.6, 78.6, and 242 nM, respectively. The (*S*)-(+)-enantiomer bound to DOR and KOR with IC_{50}s of 70.6 and 78.0 nM, respectively, and more potently than morphine, but less potently at MOR with an IC_{50} of 736 nM. MT-45 (racemate) bound to MOR more potently than its (*S*)-(+) isomer at 644 nM, but less potently at DOR and KOR sites with IC_{50}s of 156 and 176 nM, respectively. The (*R*)-(−)-enantiomer bound to all three receptors less potently than either the racemate or the (*S*)-(+)-enantiomer with IC_{50}'s of 644, 156, and 176 for MOR, DOR, and KOR, respectively (Nozaki et al. 1983). Curiously, (*R*)-(−)-MT-45 binds with 1,000×-fold greater affinity (IC_{50}'s) to both the σ_1 and σ_2 receptors (1.4 nM and 1.8 nM, respectively) than that to the opioid receptor subtypes, using guinea pig brain membrane and [^{3}H]pentazocine as the radioligand (Matsuno et al. 1998). MT-45 also demonstrates in vitro functional activity, whereby its (*S*)-(+)- and (*R*)-(−)-isomers inhibit stimulation of the guinea pig ileum with IC_{50}s of 0.0153, 0.0127, and 0.107 μM, respectively (Fujimura et al. 1978).

MT-45 produces many effects characteristic of morphine-like compounds including antinociception, gastrointestinal inhibition, Straub tail, and respiratory depression. Nakamura and colleagues conducted extensive analgesia tests with MT-45 and its enantiomers in mice and rats (Nakamura and Shimizu 1976). In mice, MT-45 has analgesic activity in thermal, mechanical, electrical, and chemical pain models with ED_{50} values of 3.09, 2.15, 1.54, and 2.24 mg/kg (sc), respectively, and these potencies were similar to that of morphine except for chemical pain for which it was less potent. Similarly, MT-45 possesses analgesic activity in rats, with ED_{50} values of 6.62 and 0.73 mg/kg (sc) in thermal and mechanical pain models, respectively. The (*S*)-(+)-isomer is generally more potent than either the racemate or the (*R*)-(−)-isomer for producing antinociception in both mice and rats with magnitudes of difference dependent upon the route of administration and pain model. As with most morphine-like compounds, MT-45 reduces GI transit time in mice with potency slightly less than that of morphine (Nakamura and Shimizu 1976). The (*S*)-(+)-isomer is more potent than the racemate, and nearly 10× more potent than

the (*R*)-(−)-isomer in doing so (Nakamura and Shimizu 1976). The racemate and the (*S*)-(+)-isomer, but not the (*S*)-(−)-isomer, also produce a characteristic morphine-like Straub tail in rodents (Nakamura and Shimizu 1976). Also consistent with morphine-like drugs, the racemate and the (*S*)-(+) isomer cause respiratory depression (in anesthetized rabbits); however, the (*R*)-(−)-isomer is without effect up to 5 mg/kg (Nakamura and Shimizu 1976). In contrast to the many morphine-like effects MT-45 can produce, neither the racemate nor its isomers have effects on pupil size (in rabbits) (Nakamura and Shimizu 1976).

No published reports of controlled studies on the pharmacology of MT-45 using human subjects could be identified. Previously, Siddiqi and colleagues canvassed the literature in June of 2014 and reported that, "There have been no formal studies in humans assessing MT-45 as a potential analgesic or to determine its pharmacology" (Siddiqi et al. 2015).

3.3 Evidence of Abuse

The first instance in which MT-45 was reported as an abused drug was in Japan in 2013 (Uchiyama et al. 2014a). Since then, its availability has broadened. Subsequently, Sweden reported 28 seizures of MT-45 during an EMCDDA–Europol survey that concluded May 28, 2014 (EMCDDA–Europol 2014). In 26 of these 28 seizures, MT-45 was seized as a white/off-white powder in quantities ranging from 0.1 to 49.9 g. In this survey, Sweden reported 33 detections in which MT-45 was confirmed in biological samples (EMCDDA–Europol 2014). These biological samples were associated with 33 patients having serious adverse events, including 21 deaths. An Internet snapshot study conducted in May of 2014 by Siddiqi and colleagues found MT-45 readily available on 17 Internet sites selling MT-45 openly (Siddiqi et al. 2015). These researchers found that the most commonly reported route of use of MT-45 by users to be oral ingestion (13 user reports), followed by nasal insufflation (4 reports), then inhalation (2 reports), and rectal insertion (1 report) (Siddiqi et al. 2015). The rectal route of administration deserves further comment. One report by a user on the Erowid user website (https://www.erowid.org) explained, "I've experimented with several ROAs, including smoked, oral, intranasal and intrarectal (though not intravenous/intramuscular injection). I've found that intrarectal administration is ideal, if I use a proper needleless syringe and go about it right. There's an immediate euphoric rush which doesn't exist for other ROA's I've tried, and it uses less material, which is always a massive boon when it comes to opioids" (Anonymous 2015).

In September of 2014, a European-level risk assessment was conducted on MT-45. During a 9-month period following October 2013, MT-45 was detected (analytically confirmed) in 28 deaths and 12 non-fatal intoxications in Sweden. In 19 of the deaths, MT-45 was either reported as the cause of death or contributing to death (EMCDDA 2015). In 17 of the mortality cases, MT-45 was found in combination with at least 1 other psychoactive substance, including controlled substances, new psychoactive substances, and medicines; in the remaining 4 cases, no other

substances were detected (EMCDDA–Europol 2014). Based upon communications to the EMCDDA from the US Immigration and Customs Enforcement's Homeland Security Investigations, it was reported that a male and a female died in New York from acute intoxication with MT-45 and a combination of MT-45 and ethanol in August of 2013 (EMCDDA–Europol 2014). More recent deaths continue to be reported with MT-45 (e.g., Fels et al. 2017).

In nine MT-45-positive patients included in the STRIDA project in Sweden (see above), most presented with opioid-like symptoms, including seven with respiratory depression and decreased consciousness, five of which were deeply unconscious, two of whom were awake on admission to hospital but became unconscious in the emergency room (Helander et al. 2014). Apnea was documented in two cases and another two had cyanosis. Miosis was notable in three patients. Four patients had paresthesia in hands and feet, difficulties to grip and hand coordination, balance disturbances, and vision impairments (e.g., blurred and double vision). Although improvements were achieved during hospital care, symptoms still were reported to persist upon discharge (Helander et al. 2014). The competitive opioid receptor antagonist, naloxone, was administered to seven of these nine patients of which four responded well. In three patients, a low oxygen saturation and/or depressed respiratory rate exceeding 24 h was recorded (Helander et al. 2014). Three of these nine patients complained of bilateral hearing loss that persisted for over 2 weeks (Helander et al. 2014). Helander and colleagues have noted that, "Ototoxicity is a very infrequent adverse reaction to chronic heavy consumption or acute overdose of opioids, reported in rare cases of heroin, methadone, oxymorphone, or hydrocodone/acetaminophen intake" (Helander et al. 2014).

Because of its emerging abuse and harms caused by its use, several countries in Europe and North America have brought MT-45 under regulatory control, and following a recommendation by the WHO Expert Committee on Drug Dependence, in 2016, the UN Commission on Narcotic Drugs voted in favor to include MT-45 under international control regulated by the Single Convention on Narcotic Drugs, 1961 (CND Blog 2016).

4 Acetylfentanyl

4.1 Chemistry and Detection

Acetylfentanyl belongs to the 4-anilinopiperidine class of synthetic opioids that also includes fentanyl. Acetylfentanyl is an acetamide derivative of 4-anilinopiperidine whereas fentanyl is a propionamide. Desmethyl fentanyl is a synonym for acetylfentanyl, due to the removal of a methyl group from the structure of fentanyl. Acetylfentanyl can be identified as an impurity during the production or degradation of fentanyl (Garg et al. 2010).

4.1.1 Synthesis

One synthetic path for acetylfentanyl is outlined in Fig. 10 as a three-step strategy resulting in a high-yield transformation (Valdez et al. 2014). It begins with the alkylation of commercially available 4-piperidone monohydrate hydrochloride **1** with 2-(bromoethyl)benzene in the presence of cesium carbonate to furnish alkylated piperidone **2**. Reductive amination of **2** using an aniline mediated by sodium triacetoxyborohydride in the presence of acetic acid yielded the 4-anilino-*N*-phenethylpiperidine precursor **3** (4-ANPP). Lastly, treatment of **3** with acetyl chloride in the presence of Hunig's base provides acetylfentanyl **4**. Due to the simplicity of its preparation, it is likely that it could also be prepared under nonindustry-level environments (Fig. 11).

4.1.2 Forensic Detection

The specific detection of acetylfentanyl remains elusive when using traditional screening techniques (e.g., ELISA), and cross-reactivity is commonly observed due to structural similarity with fentanyl. However, for unambiguous identification,

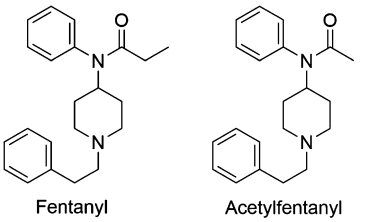

Fig. 10 Structures of fentanyl and acetylfentanyl

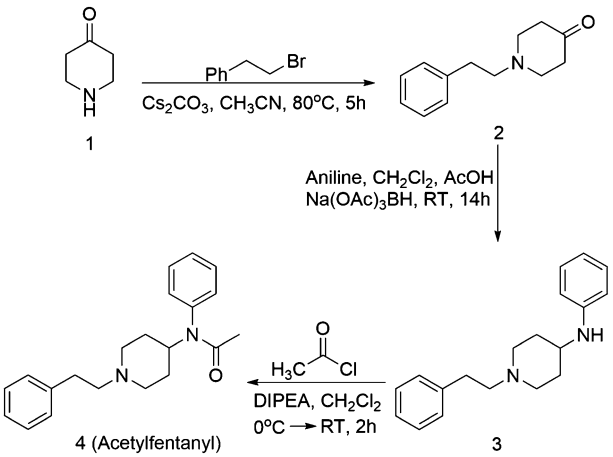

Fig. 11 Synthetic route for acetylfentanyl

confirmatory analyses involving chromatographic and mass spectrometric detection methods are needed (Runagyuttikarn et al. 1990; Wang et al. 2014; Katselou et al. 2016; Cunningham et al. 2016). General forensic analytical guidelines for detection of acetylfentanyl have been provided in a US Center for Disease Control and Prevention Health Advisory alert (CDC 2013), which included ions for routine acid/base liquid-liquid extractions followed by analysis with GC-MS for blood samples (Lozier et al. 2015). Other approaches have been reported (Ohta et al. 1999), including an assay that coupled solid phase extraction (SPE) with LC-MS/MS for detecting acetylfentanyl and its metabolite acetylnorfentanyl in urine (Patton et al. 2014).

4.2 Pharmacology

Acetylfentanyl displaces [^{3}H]etorphine binding in rat cerebral membrane with an EC_{50} of 676 nM (Woods et al. 1988). By comparison, morphine's EC_{50} is 23.6 nM (Woods et al. 1988). Acetylfentanyl completely inhibits the mouse vas deferens stimulated twitch with an EC_{50} of 442 nM (Woods et al. 1988). This inhibition is unaffected by the DOR antagonist, ICI-17864, but is reduced to 29.3 ($\pm$2.1)% levels by the MOR antagonist, beta-funaltrexamine, and is reversed by an equimolar concentration of naltrexone (Woods et al. 1988).

Aceto and colleagues reported antinociceptive effects of acetylfentanyl in mice using the tail flick and the phenylquinone writhing tests (Aceto et al. 1988). The ED_{50} (95% C.I.) for acetylfentanyl in the tail flick test was 0.3 (0.2–0.5) mg/kg (sc). As a comparator, morphine was 19$\times$ less potent at 5.8 (5.7–5.9) mg/kg (sc). In the phenylquinone writhing test, acetylfentanyl had an ED_{50} of 0.05 (0.03–0.1) mg/kg (sc), and again morphine was less potent when compared at an ED_{50} of 0.23 (0.2–0.25) mg/kg (sc) (Aceto et al. 1988). Acetylfentanyl also demonstrated antinociceptive activity in the acetic acid writhing test in ddy-mice with an ED_{50} of 0.021 mg/kg p.o. (Higashikawa and Suzuki 2008). Acetylfentanyl was 15.7$\times$ more potent in this test than morphine that had an ED_{50} of 0.33 mg/kg, but with 0.29$\times$ the potency of fentanyl that had an ED_{50} of 0.0061 mg/kg (Higashikawa and Suzuki 2008). The ratio of acetylfentanyl's LD_{50}/ED_{50} was 442.9, and far less than morphine's at 1,424.2 or fentanyl's at 10,163.9 (Higashikawa and Suzuki 2008), suggesting a far narrower safety index for acetylfentanyl than for these other opioids.

Acetylfentanyl has been tested for its ability to attenuate signs of spontaneous withdrawal in morphine-dependent rhesus monkeys (Aceto et al. 1988). Rhesus monkeys were made dependent upon morphine by four daily injections of 3 mg/kg (sc) morphine during several weeks of treatment. After morphine administration was discontinued for 15 h, the monkeys began to show signs of spontaneous withdrawal. Readministering the 3 mg/kg morphine dose completely suppressed and prevented further signs of withdrawal from emerging during the 150 min test session. Acetylfentanyl was tested at 0.125 and 0.5 mg/kg; at 0.5 mg/kg (sc), it completely, but briefly (90 min), substituted for morphine and suppressed withdrawal signs. At peak effect, the drug was considered to be 6$\times$ more potent than

morphine. Demonstrating an ability to attenuate signs of morphine withdrawal indicates cross-dependency between morphine and acetylfentanyl and suggests that opiate-dependent individuals, such as those dependent upon heroin, would find relief during withdrawal by administering acetylfentanyl and could subsequently seek it out if only for that effect.

4.3 Evidence of Abuse

Acetylfentanyl has been sold on the Internet and often as a "research chemical." For instance, as early as 2013, acetylfentanyl was identified in herbal products obtained over the Internet in Japan (Uchiyama et al. 2014b). The products appeared as alternatives to controlled substances such as narcotics and designated substances (Uchiyama et al. 2014b).

Acetylfentanyl has been detected in tablets that mimic pharmaceutical opiate products, in powder form and spiked on blotter papers (U.S. Department of Justice 2015). The first laboratory submission of acetylfentanyl in the United States was recorded in Maine in April 2013 according to the NFLIS (DEA 2015). The incidence of samples identified as containing acetylfentanyl grew in the United States from 8 in 2013, to 59 confirmations in 2014, to 1,669 confirmations in 2016 (DEA 2015, 2017).

In Europe, the EMCDDA reported that acetylfentanyl has been identified as being used or found in seizures in Finland, Belgium, Slovakia, Germany, France, Poland, Sweden, Norway, and the United Kingdom (EMCDDA–Europol 2016). The first notification occurred in 2014 by Poland in which acetylfentanyl was identified in a 20.2 g seizure of white powder by Polish customs (EMCDDA–Europol 2016). In several seizures in Europe, China has been identified as the source, and in one instance, a large shipment of 103.57 g of acetylfentanyl was being sent to a private person in France (EMCDDA–Europol 2016).

In late April 2013, police raided seven locations in Montreal seizing more than 300,000 tablets of illegally produced synthetic prescription drugs (CBC News 2013), 11,000 of these pills contained acetylfentanyl (DEA 2015). In addition, 3 kg of acetylfentanyl in powder form was confiscated (DEA 2015). Given that a typical dose of acetylfentanyl is in the microgram range, a 3 kg quantity could potentially produce millions of dosage units (DEA 2015). This was the first time Montreal police had confiscated acetylfentanyl on the black market (CBC News 2013).

The US Drug Enforcement Administration reported in July of 2015 that at least 52 confirmed fatalities involving acetylfentanyl in the United States had occurred between 2013 and 2015. Among the earliest fatalities reported in the United States involving acetylfentanyl occurred in the state of Rhode Island between March and May of 2013 (CDC 2013; Lozier et al. 2015; Ogilvie et al. 2013), ten fatalities attributed to acetylfentanyl overdose were reported during March of 2013 alone in Rhode Island (Ogilvie et al. 2013; CDC 2013; Lozier et al. 2015). Subsequently,

between March and May of 2013, four additional deaths attributed to acetylfentanyl overdose were reported in Rhode Island (Lozier et al. 2015; CDC 2013). Ages ranged from 19 to 59 years, with 10 of the 14 decedents being male (Lozier et al. 2015). Eight decedents tested positive for both acetylfentanyl and the 4-ANPP precursor (Lozier et al. 2015). Five tested positive for acetylfentanyl (no 4-ANPP), and one tested positive for 4-ANPP without acetylfentanyl (Lozier et al. 2015). Other drugs of abuse detected were cocaine (57%), morphine (36%), ethanol (36%), and benzodiazepines (21%) (Lozier et al. 2015). One decedent tested positive for acetylfentanyl and none of these other drugs (Lozier et al. 2015). Fatalities associated with acetylfentanyl use have been confirmed in several other states including Arizona, Florida, Massachusetts, New Hampshire, Vermont, New Jersey, California, Louisiana, Maryland, North Carolina, Oregon, Pennsylvania, and Wisconsin (U.S. Department of Justice 2015; Isenschmid et al. 2014). In 1 report that reviewed 18 of these fatalities, the average acetylfentanyl whole blood concentration was 160 ng/mL (range, 0.58–730 ng/mL) (Isenschmid et al. 2014). All of these 18 decedents were male except 1 female (1 not reported), and the average age was 33 years (15 reporting with a range of 19–54 years). For the cases where full toxicology was performed, both new psychoactive substances (NPS) and more traditional drugs were also identified including phenazepam (2), 4-methylethcathinone (1), methylone (1), synthetic cannabinoid receptor agonists (6), benzodiazepines (5), alprazolam (2), antidepressants/antipsychotics (5), opiates (2), cocaine (2), diphenhydramine (1), and methamphetamine (1). Additionally, two cases were also positive for fentanyl, one at a high (22 ng/mL) and one at a low (0.67 ng/mL) concentration (Isenschmid et al. 2014). As mentioned above for the deaths in Rhode Island (CDC 2013), there have been other reports from states where the only drug that could be detected in a fatality was acetylfentanyl (McIntyre et al. 2015). Deaths attributable to or at least associated with acetylfentanyl continues unabated in the United States; in just four counties in Southwestern Pennsylvania, a total of 41 opioid overdose deaths were reported between January 2015 and February 2016 and, as the authors reporting this incidence remarked, "... may represent the largest reported localized cohort of acetylfentanyl related overdose deaths within the United States" (Dwyer et al. 2018).

The first death associated with acetylfentanyl reported to the EMCDDA occurred in 2013; 32 deaths had been reported by 2016 (EMCDDA–Europol 2016). In 2 of these 32 deaths, acetylfentanyl was the only substance detected. In the other 30 deaths, a variety of other drugs were co-detected including benzodiazepines, other opioids, antidepressants, antipsychotics, THC, synthetic cannabinoids, cocaine, amphetamine, methamphetamine, MDMA, methylphenidate, methoxetamine, α-PVT, and MDPBP (EMCDDA–Europol 2016). Melent'ev and colleagues reported detecting acetylfentanyl associated with 12 deaths in the Russian Federation, sometimes occurring with morphine, but in the absence of other narcotic or psychotropic substances (Melent'ev et al. 2015).

Several countries in Europe including Austria, Cyprus, Estonia, Finland, Ireland, Latvia, Lithuania, Sweden, Poland, Norway, and the United Kingdom have enacted control measures at national level to disrupt distribution of acetylfentanyl, as well as in China (EMCDDA 2016). Because of the incidences of drug seizures and

associated fatalities with acetylfentanyl, the US Department of Justice placed the drug under temporary listing of substances subject to emergency scheduling in 2015 and subsequently permanently scheduled it within Schedule I in 2017 in response to its 2016 inclusion in Schedule I of the Single Convention on Narcotic Drugs, 1961, by the United Nations (CND Blog 2016).

5 Are Current Recovery Agents Adequate in Treating Synthetic Opioid Overdose?

The use of synthetic opioids has soared during the last 5 years mirrored by a corresponding number of fatalities associated with their use (Rudd et al. 2016; CDC 2016). Concurrent with this increased use, there have been increasing reports that either multiple initial doses or repeated continued administrations of naloxone in any of its various formulations have been required to treat synthetic opioid overdose cases (Faul et al. 2017; Sutter et al. 2017; Gussow 2016). Why have multiple doses of naloxone been necessary for treating overdosing by synthetic opioids? Are the synthetic opioids inherently more resistant to antagonism by naloxone relative to older morphinans? There are several possible reasons.

Principal factors that affect the ability of naloxone to reverse an opioid respiratory depressant effect include its pharmacokinetics and pharmacodynamics as well as those of the agonist, in particular the speed at which naloxone and agonist penetrate the blood-brain barrier (BBB) to get to the MOR, and the binding kinetics at the receptor of the two. Most of these factors are unknown for the newer fentanyl derivatives that have been found recently abused, and the following uses fentanyl throughout as an exemplar. The speed of access to brain of both naloxone and fentanyl are short, with blood-effect site equilibrium half-lives of $t_{1/2}k_{e0}$ of 6.5 and 5–15 min, respectively (Dahan et al. 2005; Lotsch 2005; Yassen et al. 2007). The receptor dissociation kinetics for both are also rapid, with naloxone with a $t_{1/2}k_{off} = 0.82$ min and for fentanyl with a $t_{1/2}k_{off} < 0.1$ min (Dahan et al. 2005). These rapid receptor kinetics of fentanyl result in onset and offset profile on breathing that is primarily determined by its opioid transfer to the MOR in the brainstem (Pattinson 2008; Boom et al. 2012; Yassen et al. 2007). Once administered, naloxone crosses the blood-brain barrier through diffusion as well as a saturable influx transport system (Suzuki et al. 2010). Fentanyl also crosses the BBB via diffusion and via an active carrier-mediated transporter (Henthorn et al. 1999; Elkiweri et al. 2009). Both naloxone and fentanyl may be substrates for a common influx transporter because naloxone pretreatment can reduce fentanyl brain/plasma partitioning by over fourfold when presumably acting through an influx transport system (Elkiweri et al. 2009). Naloxone can inhibit fentanyl's transport through the BBB (Elkiweri et al. 2009) suggesting it is potentially a substrate for a similar transporter as is fentanyl. If a reciprocal relationship obtained, saturation of the transporter with fentanyl (or one of its analogs at high doses) could inhibit naloxone's ability to pass through the BBB and into the brain as well. This remains speculative until it is demonstrated, and

it should be noted that morphine, pentazocine, tramadol, and naltrexone do not interfere with naloxone's penetrations through the BBB via this influx transporter (Suzuki et al. 2010). Once at the receptor site, the rate-limiting factors in naloxone reversal of an opioid effect are the binding kinetics of the opioid agonist at the receptor (Boom et al. 2012), and this parameter is unknown for most of the newer fentanyl derivatives.

Naloxone's rapid receptor kinetics generally do not typically make them the rate-limiting factors governing its overall ability to reverse opioid agonist actions. For full agonists such as fentanyl, single administrations of naloxone typically can reverse fentanyl-induced respiratory depression, but this effect is dependent upon the naloxone dose and the kinetics of its particular administration (Boom et al. 2012). Regarding the kinetics of naloxone, a reason in some cases multiple doses of it are needed to revive a synthetic opioid overdosed user is the relatively short duration of action of naloxone when competing against drugs with likely much longer durations of actions. The elimination half-life of naloxone is 33 min (Yassen et al. 2007), the half-life of fentanyl is several times longer than naloxone's (Streisand et al. 1991), and the half-life of carfentanil, as one example of a fentanyl-like analog, is many times longer than that of fentanyl (Raffa et al. 2017). The duration of activities of most of the newer fentanyl analogs and other kinds of synthetic opioids have not been reported, but some are likely to be even longer than that of fentanyl as well. For instance, in recent, unpublished studies in our laboratory (P.M.B.), *ortho*-fluorofentanyl still was maintaining maximal levels of antinociception in mice when tested 2 h after its last administration at its final determination, while the level of antinociception induced by fentanyl at 10 times the *ortho*-fluorofentanyl dose had petered out to below 50% levels. The duration of action of some of the newer synthetic opioids may, in itself, dictate the need for multiple naloxone doses to prevent renarcotization.

Another likely consideration that dictates the dose(s) of naloxone needed to revive an overdose patient is that it is typically unknown what synthetic opioid, its potency, and the dose that has been taken. Considering that synthetic opioids are often used in mixtures with heroin (CDC 2018; UNODC 2017b) or with, or as posing as, clinically used opioids such as hydrocodone or oxycodone (e.g., Sutter et al. 2017), much is left to the preparer of the illicitly produced product to constitute it at an homogenous target concentration. The potencies of the synthetic opioids are often greater than conventional morphinans. Fentanyl (Finch and DeKornfeld 1967) and carfentanil (Van Bever et al. 1976), for example, are up to 100 to 10,000 times more potent than morphine, respectively. Poorly prepared, nonhomogeneous potent opioid mixtures have provided challenges to emergency medical service providers (Faul et al. 2017). In one case, pills masquerading as authentic hydrocodone/acetaminophen tablets contained up to nearly 7 mg fentanyl were confiscated from overdosed users presenting to an urban academic emergency department in California (Sutter et al. 2017) each sufficient in itself to result in toxic serum concentrations (Sutter et al. 2017; Streisand et al. 1991).

Overall, the need for multiple dosing of naloxone to initiate and maintain revival after synthetic opioid overdose is likely attributable to a combination of factors

including the potentially long duration of activity of the agonists, to their high potency that becomes especially dangerous in ill-prepared drug mixtures in which they are non-homogeneously distributed, and to parameters such as their binding kinetics at the MOR as well as their potential competition with naloxone for a common carrier-mediated transporter into the BBB.

6 Conclusions

In 2016, there were more than 19,000 deaths related to synthetic opioid overdose (excluding methadone) in the United States alone, representing a doubling from 2015 to 2016 (CDC 2016). The problem is not confined to North America. Since 2012, the EMCDDA, via the EU Early Warning System, has been receiving an increasing number of reports of synthetic opioid abuse and of harms caused by them, with 25 new opioids being detected on Europe's drug market since 2009 (EMCDDA 2017). The international community has been trying to respond to this menace. At the recent 2018, 61st regular session of the Commission on Narcotic Drugs, the Commission placed six synthetic opioids under international control following recommendations by the WHO Expert Committee on Drug Dependence (ocfentanil, furanylfentanyl (Fu-F), acryloylfentanyl (acrylfentanyl), 4-fluoroisobutyrfentanyl (para-fluoroisobutyrfentanyl, 4-FIBF, pFIBF), and tetrahydrofuranylfentanyl (THF-F), and carfentanil). Historically, however, soon after one synthetic opioid is controlled, a variant has soon replaced it. Synthetic opioids may require new levels of law enforcement and medical treatment strategies. Potency by itself of some forces a new frame of reference. For example, although only 2.7 kg of carfentanil was reported in 618 seizures by countries to the EMCDDA (EMCDDA–Europol 2017), given carfentanil's potency, this was the equivalent of ~27,000 kg of morphine. Potency, long duration of activity, and perhaps other less explored properties of the synthetic opioids may make them unusually lethal and refractory to treatment with opioid antagonists. Although most synthetic abused opioids to date have been based upon phenylpiperidine, fentanyl-like structures, benzamides and piperazines like U-47700 and MT-45, respectively, may foreshadow future cultivations.

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References

Aceto M, Bowman E, Harris L, May E (1988) Dependence studies of new compounds in the rhesus monkey, rat, and mouse, 1987. In: Problem of drug dependence, 1987: proceedings of the 49th annual scientific meeting, The Committee on Problems of Drug Dependence, Inc., vol 81. NIDA Research Monograph, vol 81. Office of Science, National Institute on Drug Abuse, Rockville, MD, pp 485–542

Anonymous (2015) A few trials: an experience with MT-45 (ID 103454). Erowid.org. https://www.erowid.org/experiences/exp.php?ID=103454. Accessed 12 Aug 2015

Armenian P, Olson A, Anaya A, Kurtz A, Ruegner R, Gerona RR (2016) Fentanyl and a novel synthetic opioid U-47700 masquerading as street "Norco" in Central California: a case report. Ann Emerg Med. https://doi.org/10.1016/j.annemergmed.2016.06.014

Barber A, Gottschlich R (1997) Novel developments with selective, non-peptidic kappa-opioid receptor agonists. Expert Opin Investig Drugs 6:1351–1368. https://doi.org/10.1517/13543784.6.10.1351

Benchikh E (2017) Immunodetection of MT-45, a synthetic opioid drug of abuse. US Patent 20170343570

Boom M, Niesters M, Sarton E, Aarts L, Smith TW, Dahan A (2012) Non-analgesic effects of opioids: opioid-induced respiratory depression. Curr Pharm Des 18:5994–6004. https://doi.org/10.2174/138161212803582469

Brittain RT, Kellett DN, Neat ML, Stables R (1973) Anti-nociceptive effects in N-substituted cyclohexylmethylbenzamides. Br J Pharmacol 49:P158–P159

Casy AF, Parfitt RT (1986) Opioid analgesics: chemistry and receptors. Springer Science & Business Media, New York

CBC News (2013) Extremely potent painkiller hits Montreal black market: desmethyl fentanyl is 40 times more potent than heroin. CBC News. http://www.cbc.ca/news/canada/montreal/extremely-potent-painkiller-hits-montreal-black-market-1.1340808. Accessed 27 Aug 2015

CDC (Centers for Disease Control and Prevention) (2013) Acetyl fentanyl overdose fatalities – Rhode Island, March–May 2013. MMWR Morb Mortal Wkly Rep 62(34):703–704

CDC (Centers for Disease Control and Prevention) (2017) Understanding the epidemic. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, Division of Unintentional Injury Prevention. https://www.cdc.gov/drugoverdose/epidemic/index.html. Accessed 8 Apr 2018

CDC (Centers for Disease Control and Prevention), National Center for Injury Prevention and Control, Division of Unintentional Injury Prevention (2016) Synthetic opioid data. Centers for Disease Control and Prevention. https://www.cdc.gov/drugoverdose/data/fentanyl.html. Accessed 20 Feb 2018

CDC (Centers for Disease Control and Prevention) NCfIPaC, Division of Unintentional Injury Prevention (2018) Fentanyl. Centers for Disease Control and Prevention. https://www.cdc.gov/drugoverdose/opioids/fentanyl.html. Accessed 16 Feb 2018

Cheney BV, Szmuszkovicz J, Lahti RA, Zichi DA (1985) Factors affecting binding of trans-N-[2-(methylamino)cyclohexyl]benzamides at the primary morphine receptor. J Med Chem 28:1853–1864

CND Blog (2016) Item 6b: changes in the scope of control. Live reporting from the UN Commission on Narcotic Drugs Agenda, vol 2018. cndblog.org, http://cndblog.org/2016/03/plenary-9th-meeting-item-6-implementation-of-the-international-drug-control-treaties/

Collins RJ, Kaplan LJ, Ludens JH, Von Voigtlander PF (1984) Oxygen substituted amino-cyclohexyl-benzeneacetamides and-benzamides as water diuretic drugs. United States Patent 4463013A

Commission on Narcotic Drugs (2015) Report on the fifty-eighth session (5 December 2014 and 9–17 March 2015) (trans: Official Records of the Economic and Social Council). New York. http://www.undocs.org/E/2015/28

Coopman V, Blanckaert P, Van Parys G, Van Calenbergh S, Cordonnier J (2016) A case of acute intoxication due to combined use of fentanyl and 3,4-dichloro-N- 2-(dimethylamino) cyclohexyl-N-methylbenzamide (U-47700). Forensic Sci Int 266:68–72. https://doi.org/10.1016/j.forsciint.2016.05.001

Cunningham SM, Haikal NA, Kraner JC (2016) Fatal intoxication with acetyl fentanyl. J Forensic Sci 61:S276–S280. https://doi.org/10.1111/1556-4029.12953

Dahan A, Yassen A, Bijl H, Romberg R, Sarton E, Teppema L, Olofsen E, Danhof M (2005) Comparison of the respiratory effects of intravenous buprenorphine and fentanyl in humans and rats. Br J Anaesth 94:825–834. https://doi.org/10.1093/bja/aei145

DEA (Drug Enforcement Administration) (2015) Acetyl fentanyl: background information and evaluation of 'three factor analysis' (factors 4, 5 and 6) for temporary scheduling Drug and Chemical Evaluation Section, Office of Diversion Control, Drug Enforcement Administration, Springfield, VA

DEA (United States Drug Enforcement Administration) (2016a) Counterfeit prescription pills containing fentanyls: a global threat. DEA Intelligence Brief. DEA-DCT-DIB-021-16. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0ahUKEwjOkvzc6enaAhWGt1kKHT9dDtsQFggpMAA&url=https%3A%2F%2Fwww.dea.gov%2Fdocs%2FCounterfeit%2520Prescription%2520Pills.pdf&usg=AOvVaw1o62uLCCi5ySV2yjv1Eu-J

DEA (Drug Enforcement Administration) (2016b) Schedules of controlled substances: temporary placement of U-47700 into schedule I. Billing code 4410-09-P, vol Billing code 4410-09-P. https://www.federalregister.gov/documents/2016/09/07/2016-21477/schedules-of-controlled-substances-temporary-placement-of-u-47700-into-schedule-i

DEA (Drug Enforcement Administration, Diversion Control Division) (2017) NFLIS brief: fentanyl and fentanyl-related substances reported in NFLIS, 2015–2016. US Drug Enforcement Administration, Springfield. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0ahUKEwjT3fGZ4OnaAhVSGt8KHTmjAcgQFggpMAA&url=https%3A%2F%2Fwww.nflis.deadiversion.usdoj.gov%2FDesktopModules%2FReportDownloads%2FReports%2F11350_R1_NFLIS_Research_Brief_Fentanyl.pdf&usg=AOvVaw2hlzPE8DKknBW-9LQ-OfY

Domanski K, Kleinschmidt KC, Schulte JM, Fleming S, Frazee C, Menendez A, Tavakoli K (2016) Two cases of intoxication with new synthetic opioid, U-47700. Clin Toxicol (Phila) 1–5. https://doi.org/10.1080/15563650.2016.1209763

Dwyer JB, Janssen J, Luckasevic TM, Williams KE (2018) Report of increasing overdose deaths that include acetyl fentanyl in multiple counties of the southwestern region of the commonwealth of Pennsylvania in 2015-2016. J Forensic Sci 63:195–200. https://doi.org/10.1111/1556-4029.13517

ElectronicExorcist (2016) I just got some U-47700 – AMA. reddit.com/RCSOURCES. https://www.reddit.com/r/RCSources/comments/34g78f/i_just_got_some_u47700_ama/. Accessed 28 Aug 2016

Elkiweri IA, Zhang YL, Christians U, Ng KY, Tissot van Patot MC, Henthorn TK (2009) Competitive substrates for P-glycoprotein and organic anion protein transporters differentially reduce blood organ transport of fentanyl and loperamide: pharmacokinetics and pharmacodynamics in Sprague-Dawley rats. Anesth Analg 108:149–159. https://doi.org/10.1213/ane.0b013e31818e0bd1

Ellefsen KN, Taylor EA, Simmons P, Willoughby V, Hall BJ (2017) Multiple drug-toxicity involving novel psychoactive substances, 3-fluorophenmetrazine and U-47700. J Anal Toxicol 41:765–770. https://doi.org/10.1093/jat/bkx056

Elliott SP, Brandt SD, Smith C (2016) The first reported fatality associated with the synthetic opioid 3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide (U-47700) and implications for forensic analysis. Drug Test Anal 8:875–879. https://doi.org/10.1002/dta.1984

EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) (2015) European drug report: trends and developments. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Luxembourg. doi: 10.2810/084165. ISBN: 978-92-9168-776-3 http://www.emcdda.europa.eu/system/files/publications/974/TDAT15001ENN.pdf

EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) (2016) EU early warning system alert: U-47700 in Europe. EU-EWS-RCS-AL-2016-0003. EMCDDA, Lisbon

EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) (2017) European drug report 2017: trends and developments. Publications Office of the European Union, Luxembourg

EMCDDA–Europol (2016) EMCDDA–Europol joint report on a new psychoactive substance: N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]acetamide (acetylfentanyl). Joint reports, Luxembourg

EMCDDA–Europol (2017) EMCDDA–Europol joint report on a new psychoactive substance: methyl 1-(2-phenylethyl)-4-[phenyl(propanoyl)amino]piperidine-4-carboxylate (carfentanil). European Monitoring Centre for Drugs and Drug Addiction, Luxembourg

EMCDDA–Europol (2014) EMCDDA–Europol joint report on a new psychoactive substance: 1-cyclohexyl-4-(1,2-diphenylethyl)piperazine ('MT-45'). EMCDDA–Europol joint report. European Monitoring Centre for Drugs and Drug Addiction, Luxembourg

Faul M, Lurie P, Kinsman JM, Dailey MW, Crabaugh C, Sasser SM (2017) Multiple naloxone administrations among emergency medical service providers is increasing. Prehosp Emerg Care 21:411–419. https://doi.org/10.1080/10903127.2017.1315203

Fels H, Krueger J, Sachs H, Musshoff F, Graw M, Roider G, Stoever A (2017) Two fatalities associated with synthetic opioids: AH-7921 and MT-45. Forensic Sci Int 277:e30–e35. https://doi.org/10.1016/j.forsciint.2017.04.003

Finch JS, DeKornfeld TJ (1967) Clinical investigation of the analgesic potency and respiratory depressant activity of fentanyl, a new narcotic analgesic. J Clin Pharmacol J New Drugs 7:46–51. https://doi.org/10.1002/j.1552-4604.1967.tb00029.x

Fleming SW, Cooley JC, Johnson L, Frazee CC, Domanski K, Kleinschmidt K, Garg U (2017) Analysis of U-47700, a novel synthetic opioid, in human urine by LC-MS-MS and LC-QToF. J Anal Toxicol 41:173–180. https://doi.org/10.1093/jat/bkw131

Fujimura H, Kawai K (1960) Diphenylalkylamine derivatives. I. Comparative studies of the optical isomers of 1,2-diphenyl-dimethylaminoethane hydrochloride. Nippon Yakurigaku Zasshi 56:514

Fujimura H, Tsurumi K, Nozaki M, Hori M, Imai E (1978) Analgesic activity and opiate receptor binding of 1-cyclohexyl-4-(1,2-diphenylethyl)piperazine. Jpn J Pharmacol 28:505–506

Garg A, Solas DW, Takahashi LH, Cassella JV (2010) Forced degradation of fentanyl: identification and analysis of impurities and degradants. J Pharm Biomed Anal 53:325–334. https://doi.org/10.1016/j.jpba.2010.04.004

Gussow L (2016) Toxicology rounds: who said the opioid crisis couldn't get any worse? Emerg Med News 38:1,29–1,30. https://doi.org/10.1097/01.Eem.0000508281.75514.70

Harding SA, Biary R, Hoffman RS, Su MK, Cooper GA, Smith SW (2017) Novios muertos: two confirmed fatalities from U-47700. Clin Toxicol (Phila) 55:449–449

Harper NJ, Veitch GBA (1976) 1-(3,4-dichlorobenzamidomethyl)-cyclohexyldimethylamine. US Patent 3975443A

Harper NJ, Veitch GBA (1977) Ethylene diamine derivatives. US Patent 4049663A

Helander A, Backberg M (2016) New psychoactive substances (NPS) – the hydra monster of recreational drugs. Clin Toxicol (Phila) 55:1–3. https://doi.org/10.1080/15563650.2016.1217003

Helander A, Backberg M, Beck O (2014) MT-45, a new psychoactive substance associated with hearing loss and unconsciousness. Clin Toxicol (Phila) 52:901–904. https://doi.org/10.3109/15563650.2014.943908

Henthorn TK, Liu Y, Mahapatro M, Ng KY (1999) Active transport of fentanyl by the blood-brain barrier. J Pharmacol Exp Ther 289:1084–1089

Higashikawa Y, Suzuki S (2008) 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 analogs. Forensic Toxicol 26:1–5. https://doi.org/10.1007/s11419-007-0039-1

INCB (International Narcotics Control Board) (2017) List of narcotic drugs under international control. 56th edn. p 25. http://www.emcdda.europa.eu/system/files/publications/974/TDAT15001ENN.pdf

Isenschmid D, Kacinko S, Logan B (2014) Case findings in 18 acetylfentanyl related deaths. World Forensic Festival 2014. Seoul, Korea

Janowsky A (2016) U-47700: trans-3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methyl-benzamide: Binding and functional activity at delta, kappa and mu opioid receptors (trans: Research Service (R&D-22)). vol DEA-VA interagency agreement title: "In Vitro Receptor and Transporter Assays for Abuse Liability Testing for the DEA by the VA" Department of Veterans Affairs Medical Center, Portland, OR

Jesusgreen (2014) Thread: novel opioid, U-47700-Mega thread and FAQ. Bluelight.org. http://www.bluelight.org/vb/threads/739960-Novel-opioid-U-47700?highlight=47700

Jones MJ, Hernandez BS, Janis GC, Stellpflug SJ (2016) A case of U-47700 overdose with laboratory confirmation and metabolite identification. Clin Toxicol (Phila) 1–5. doi: https://doi.org/10.1080/15563650.2016.1209767

Jones MJ, Hernandez BS, Janis GC, Stellpflug SJ (2017) A case of U-47700 overdose with laboratory confirmation and metabolite identification. Clin Toxicol (Ohila) 55:55–59. https://doi.org/10.1080/15563650.2016.1209767

Kalamazoo JS (1970) Ethers of alpha-phenyl-2-aminocycloalkanemethanols. US Patent 499033A

Katselou M, Papoutsis I, Nikolaou P, Spiliopoulou C, Athanaselis S (2015) AH-7921: the list of new psychoactive opioids is expanded. Forensic Toxicol 33:195–201. https://doi.org/10.1007/s11419-015-0271-z

Katselou M, Papoutsis I, Nikolaou P, Spiliopoulou C, Athanaselis S (2016) Old opioids, new concerns: the case of acetylfentanyl. Forensic Toxicol 34:201–212. https://doi.org/10.1007/s11419-016-0310-4

Loew G, Lawson J, Toll L, Frenking G, Berzetei-Gurske I, Polgar W (1988a) Structure activity studies of two classes of beta-amino-amides: the search for kappa-selective opioids. NIDA Res Monogr 90:144–151

Loew G, Lawson J, Toll L, Frenking G, Berzetei-Gurske I, Polgar W (1988b) Structure activity studies of two classes of beta-amino-amides: the search for kappa-selective opioids. In: Paper presented at the National Institute on Drug Abuse research monograph series 90, problems of drug dependence 1988: proceeding of the 50th annual scientific meeting, The Committee on Problems of Drug Dependence, North Falmouth

Loew G, Toll L, Lawson J, Frenking G, Polgar W (1989) Opiate receptor heterogeneity: relative ligand affinities and molecular determinants of high affinity binding at different opiate receptors. Prog Clin Biol Res 289:411–432

Lotsch J (2005) Pharmacokinetic-pharmacodynamic modeling of opioids. J Pain Symptom Manag 29:S90–S103. https://doi.org/10.1016/j.jpainsymman.2005.01.012

Lozier MJ, Boyd M, Stanley C, Ogilvie L, King E, Martin C, Lewis L (2015) Acetyl fentanyl, a novel fentanyl analog, causes 14 overdose deaths in Rhode Island, March–May 2013. J Med Toxicol 11:208–217. https://doi.org/10.1007/s13181-015-0477-9

Matsuno K, Senda T, Kobayashi T, Murai M, Mita S (1998) Reduction of 4-cyclohexyl-1-[(1R)-1,2-diphenylethyl]-piperazine-induced memory impairment of passive avoidance performance by sigma 1 receptor agonists in mice. Methods Find Exp Clin Pharmacol 20:575–580

McIntyre IM, Trochta A, Gary RD, Malamatos M, Lucas JR (2015) An acute acetyl fentanyl fatality: a case report with postmortem concentrations. J Anal Toxicol 39:490–494. https://doi.org/10.1093/jat/bkv043

McIntyre IM, Gary RD, Joseph S, Stabley R (2017) A fatality related to the synthetic opioid U-47700: postmortem concentration distribution. J Anal Toxicol 41:158–160. https://doi.org/10.1093/jat/bkw124

Melent'ev AB, Kataev SS, Dvorskaya ON (2015) Identification and analytical properties of acetyl fentanyl metabolites. J Anal Chem 70:240–248. https://doi.org/10.1134/s1061934815020124

Michalson ET, Szmuszkovicz J (1989) Medicinal agents incorporating the 1,2-diamine functionality. Prog Drug Res 33:135–149

Mohr AL, Friscia M, Papsun D, Kacinko SL, Buzby D, Logan BK (2016) Analysis of novel synthetic opioids U-47700, U-50488 and furanyl fentanyl by LC-MS/MS in postmortem casework. J Anal Toxicol 40:709–717. https://doi.org/10.1093/jat/bkw086

Mullins DD (1966) N-(1-Aminocyclohexylmethyl) anilines and N-(1-nitrocyclohexylmethyl) anilines. US Patent US3258489A

Nakamura H, Shimizu M (1976) 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 221:105–121

Natsuka K, Nakamura H, Uno H, Umemoto S (1975) Studies on 1-substituted 4-(1,2-diphenylethyl) piperazine derivatives and their analgesic activities. 1. J Med Chem 18(12):1240–1244. https://doi.org/10.1021/Jm00246a014

Natsuka K, Nakamura H, Nishikawa Y, Negoro T, Uno H, Nishimura H (1987) Synthesis and structure-activity relationships of 1-substituted 4-(1,2-diphenylethyl)piperazine derivatives having narcotic agonist and antagonist activity. J Med Chem 30:1779–1787

Nikolaou P, Katselou M, Papoutsis I, Spiliopoulou C, Athanaselis S (2017) U-47700. An old opioid becomes a recent danger. Forensic Toxicol 35:11–19. https://doi.org/10.1007/s11419-016-0347-4

Nozaki M, Niwa M, Imai E, Hori M, Fujimura H (1983) (1,2-diphenylethyl) piperazines as potent opiate-like analgesics – the unusual relationships between stereoselectivity and affinity to opioid receptor. Life Sci 33:431–434. https://doi.org/10.1016/0024-3205(83)90534-9

O'Donnell JK, Halpin J, Mattson CL, Goldberger BA, Gladden RM (2017) Deaths involving fentanyl, fentanyl analogs, and U-47700 – 10 States, July–December 2016. MMWR Morb Mortal Wkly Rep 66:1197–1202. https://doi.org/10.15585/mmwr.mm6643e1

Ogilvie L, Stanley C, Lewis L, Boyd M, Lozier M (2013) Acetyl fentanyl overdose fatalities – Rhode Island, March–May 2013. MMWR Morb Mortal Wkly Rep 62:703–704

Ohta H, Suzuki S, Ogasawara K (1999) Studies on fentanyl and related compounds IV. Chromatographic and spectrometric discrimination of fentanyl and its derivatives. J Anal Toxicol 23:280–285. https://doi.org/10.1093/jat/23.4.280

Papsun D, Krywanczyk A, Vose JC, Bundock EA, Logan BK (2016) Analysis of MT-45, a novel synthetic opioid, in human whole blood by LC-MS-MS and its identification in a drug-related death. J Anal Toxicol 40:313–317. https://doi.org/10.1093/jat/bkw012

Pattinson KT (2008) Opioids and the control of respiration. Br J Anaesth 100:747–758. https://doi.org/10.1093/bja/aen094

Patton AL, Seely KA, Pulla S, Rusch NJ, Moran CL, Fantegrossi WE, Knight LD, Marraffa JM, Kennedy PD, James LP, Endres GW, Moran JH (2014) Quantitative measurement of acetyl fentanyl and acetyl norfentanyl in human urine by LC-MS/MS. Anal Chem 86:1760–1766. https://doi.org/10.1021/ac4036197

Police Scotland (2016a) Drug trend bulletin – issue 8, March 2016. NHS Borders, Roxburghshire

Police Scotland (2016b) Drug trend bulletin – issue 9 May 2016. NHS Borders, Roxburghshire
Prekupec MP, Mansky PA, Baumann MH (2017) Misuse of novel synthetic opioids: a deadly new trend. J Addict Med 11:256–265. https://doi.org/10.1097/ADM.0000000000000324
Raffa RB, Pergolizzi JV Jr, Le Quang JA, Taylor R Jr, NEMA Research Group, Colucci S, Annabi MH (2017) The fentanyl family: a distinguished medical history tainted by abuse. J Clin Pharm Ther 43:154–158. https://doi.org/10.1111/jcpt.12640
Roll W (1974) N-Cyclopentyl-N-2-hydroxyalkyl-ring-substituted benzamides. US Patent 3825595A
Ruan X, Kaye AD, Chiravuri S (2016) Comparing fatal cases involving U-47700. Forensic Sci Med Pathol 12:369–371. https://doi.org/10.1007/s12024-016-9795-8
Rudd RA, Seth P, David F, Scholl L (2016) Increases in drug and opioid-involved overdose deaths – United States, 2010–2015. MMWR Morb Mortal Wkly Rep 65:1445–1452. https://doi.org/10.15585/mmwr.mm655051e1
Runagyuttikarn W, Law MY, Rollins DE, Moody DE (1990) Detection of fentanyl and its analogs by enzyme-linked-immunosorbent-assay. J Anal Toxicol 14:160–164
Rynbrandt RH, Skaletzky LL (1972) Cycloalkanecarboxamides. US Patent 3647804A
Schneir A, Metushi IG, Sloane C, Benaron DJ, Fitzgerald RL (2016) Near death from a novel synthetic opioid labeled U-47700: emergence of a new opioid class. Clin Toxicol (Phila) 55:51–54. https://doi.org/10.1080/15563650.2016.1209764
Seither J, Reidy L (2017) Confirmation of carfentanil, U-47700 and other synthetic opioids in a human performance case by LC-MS-MS. J Anal Toxicol 41:493–497. https://doi.org/10.1093/jat/bkx049
Seth P, Scholl L, Rudd RA, Bacon S (2018) Overdose deaths involving opioids, cocaine, and psychostimulants – United States, 2015-2016. MMWR Morb Mortal Wkly Rep 67:349–358. https://doi.org/10.15585/mmwr.mm6712a1
Shoff EN, Zaney ME, Kahl JH, Hime GW, Boland DM (2017) Qualitative identification of fentanyl analogs and other opioids in postmortem cases by UHPLC-ion trap-MS^n. J Anal Toxicol 41:484–492. https://doi.org/10.1093/jat/bkx041
Siddiqi S, Verney C, Dargan P, Wood DM (2015) Understanding the availability, prevalence of use, desired effects, acute toxicity and dependence potential of the novel opioid MT-45. Clin Toxicol (Phila) 53:54–59. https://doi.org/10.3109/15563650.2014.983239
Spargo EA (2016) Two fatalities involving the use of the synthetic opioid U-47700. ToxTalk 40:9–13
Streisand JB, Varvel JR, Stanski DR, Lemaire L, Ashburn MA, Hague BI, Tarver SD, Stanley TH (1991) Absorption and bioavailability of oral transmucosal fentanyl citrate. Anesthesiology 75:223–229. https://doi.org/10.1097/00000542-199108000-00009
Sutter ME, Gerona RR, Davis MT, Roche BM, Colby DK, Chenoweth JA, Adams AJ, Owen KP, Ford JB, Black HB, Albertson TE (2017) Fatal fentanyl: one pill can kill. Acad Emerg Med 24:106–113. https://doi.org/10.1111/acem.13034
Suzuki T, Ohmuro A, Miyata M, Furuishi T, Hidaka S, Kugawa F, Fukami T, Tomono K (2010) Involvement of an influx transporter in the blood-brain barrier transport of naloxone. Biopharm Drug Dispos 31:243–252. https://doi.org/10.1002/bdd.707
Szmuszkovicz J (1970) 2-Anilino and 2-anilinomethyl cycloalkylamines. US Patent 3510492A
Szmuszkovicz J (1999) U-50,488 and the kappa receptor: a personalized account covering the period 1973 to 1990. Prog Drug Res 52:167–195
Szmuszkovicz J, Von Voigtlander PF (1982) Benzeneacetamide amines: structurally novel non-mu opioids. J Med Chem 25:1125–1126
Tsibulnikov SY, Maslov LN, Mukhomedzyanov AV, Krylatov AV, Tsibulnikova MR, Lishmanov YB (2015) Prospects of using of kappa-opioid receptor agonists U-50,488 and ICI 199,441 for improving heart resistance to ischemia/reperfusion. Bull Exp Biol Med 159:718–721. https://doi.org/10.1007/s10517-015-3057-8

U.S. Department of Justice, Drug Enforcement Administration, Office of Diversion Control, Drug & Chemical Evaluation Section (2015) Acetyl fentanyl (N-(1-phenethylpiperidin-4-yl)-N-phenylacetamide). http://www.deadiversion.usdoj.gov/ drug_chem_info/acetylfentanyl.pdf

Uchiyama N, Matsuda S, Kawamura M, Kikura-Hanajiri R, Goda Y (2014a) Identification of two new-type designer drugs, piperazine derivative MT-45 (I-C6) and synthetic peptide Noopept (GVS-111), with synthetic cannabinoid A-834735, cathinone derivative 4-methoxy-alpha-PVP, and phenethylamine derivative 4-methylbuphedrine from illegal products. Forensic Toxicol 32:9–18. https://doi.org/10.1007/s11419-013-0194-5

Uchiyama N, Shimokawa Y, Kawamura M, Kikura-Hanajiri R, Hakamatsuka T (2014b) Chemical analysis of a benzofuran derivative, 2-(2-ethylaminopropyl)benzofuran (2-EAPB), eight synthetic cannabinoids, five cathinone derivatives, and five other designer drugs newly detected in illegal products. Forensic Toxicol 32:266–281. https://doi.org/10.1007/s11419-014-0238-5

UNODC (United Nations Office on Drugs and Crime) (2016) February 2016 – Belgium: death caused by use of fentanyl and U-47700. https://www.unodc.org/LSS/Announcement/Details/e0417b5a-ea74-4d54-ae35-e3ad9f700e19. Accessed 3 Sept 2016

UNODC (United Nations Office on Drugs and Crime) (2017a) World drug report 2017: executive summary, conclusions and policy implications. United Nations publication, Sales No. E.17.XI.6, Vienna. https://www.unodc.org/documents/scientific/Booklet_1_Executive_Summary_conclusions_and_policy_implications.pdf

UNODC (United Nations Office on Drugs and Crime) (2017b) World drug report 2017: market analysis of synthetic drugs amphetamine-type stimulants, new psychoactive substances

Valdez CA, Leif RN, Mayer BP (2014) An efficient, optimized synthesis of fentanyl and related analogs. PLoS One 9:e108250. https://doi.org/10.1371/journal.pone.0108250

Van Bever WFM, Niemegeers CJE, Schellekens KHL, Janssen PAJ (1976) N-4-substituted 1-(2-arylethyl)-4-piperidinyl-n-phenylpropanamides, a novel series of extremely potent analgesics with unusually high safety margin. Arzneimittelforschung 26:1548–1551

Vuckovic S, Prostran M, Ivanovic M, Dosen-Micovic L, Todorovic Z, Nesic Z, Stojanovic R, Divac N, Mikovic Z (2009) Fentanyl analogs: structure-activity-relationship study. Curr Med Chem 16:2468–2474

Wang BT, Colby JM, Wu AH, Lynch KL (2014) Cross-reactivity of acetylfentanyl and risperidone with a fentanyl immunoassay. J Anal Toxicol 38:672–675. https://doi.org/10.1093/jat/bku103

WHO (World Health Organization) (2015a) Acetylfentanyl: critical review report: agenda item 5.2. In: Paper presented at the expert committee on drug dependence, thirty-seventh meeting, Geneva, 16–20 Nov 2015. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0ahUKEwj7-O3h6unaAhVjdt8KHVaKBmEQFggpMAA&url=http%3A%2F%2Fwww.who.int%2Fmedicines%2Faccess%2Fcontrolled-substances%2F5.2_Acetylfentanyl_CRev.pdf&usg=AOvVaw1pZdcaHmqeenfpIXzD8SvM

WHO (World Health Organization) (2015b) MT-45: critical review report: agenda item 5.1. In: Paper presented at the expert committee on drug dependence, thirty-seventh meeting, Geneva, 16–20 Nov 2015. http://www.who.int/medicines/access/controlled-substances/5.1_MT-45_CRev.pdf?ua=1

WHO (World Health Organization) (2016) U-47700: critical review report: agenda item 4.1. In: Paper presented at the expert committee on drug dependence thirty-eighth meeting, Geneva, 14–18 Nov 2016. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0ahUKEwi5raGE7OnaAhUPPN8KHYZvCHUQFggpMAA&url=http%3A%2F%2Fwww.who.int%2Fmedicines%2Faccess%2Fcontrolled-substances%2F4.1_U-47700_CritReview.pdf&usg=AOvVaw236aYiacg_fum0ijj0nG_V

Woods J, Medzihradsky F, Smith C, Winger G, Gmerek D (1988) Evaluation of new compounds for opioid activity: 1987 annual report. In: Problem of drug dependence, 1987: proceedings of the 49th annual scientific meeting, The Committee on Problems of Drug Dependence, Inc., vol 81. NIDA Research Monograph, vol 81. Office of Science, National Institute on Drug Abuse, Rockville, pp 543–590

Yassen A, Olofsen E, Romberg R, Sarton E, Teppema L, Danhof M, Dahan A (2007) Mechanism-based PK/PD modeling of the respiratory depressant effect of buprenorphine and fentanyl in healthy volunteers. Clin Pharmacol Ther 81:50–58. https://doi.org/10.1038/sj.clpt.6100025

Zhao S, Totleben MJ, Mizsak SA, Freeman JP, Szmuszkovicz J (2000) Phenanthridone analogs of the opiate agonist U-47,700 in the trans-1,2-diaminocyclohexane benzamide series. Heterocycles 52:325–332

Designer Benzodiazepines: Another Class of New Psychoactive Substances

Bjoern Moosmann and Volker Auwärter

Contents

Abstract

Benzodiazepines have been introduced as medical drugs in the 1960s. They replaced the more toxic barbiturates, which were commonly used for treatment of anxiety or sleep disorders at the time. However, benzodiazepines show a high potential of misuse and dependence. Although being of great value as medicines,

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dependence to these drugs is a concern worldwide, in part due to overprescription and easy availability. Therefore, the phenomenon of benzodiazepines sold via Internet shops without restrictions at low prices is alarming and poses a serious threat to public health. Most of these compounds (with the exception of, e.g., phenazepam and etizolam) have never been licensed as medical drugs in any part of the world and are structurally derived from medically used benzodiazepines. Strategies of clandestine producers to generate new compounds include typical structural variations of medically used 1,4-benzodiazepines based on structure-activity relationships as well as synthesis of active metabolites and triazolo analogs of these compounds. As they were obviously designed to circumvent national narcotics laws or international control, they can be referred to as "designer benzodiazepines." The majority of these compounds, such as diclazepam, clonazolam, and nitrazolam, have been described in scientific or patent literature. However, little is known about their pharmacological properties and specific risks related to their use. This chapter describes the phenomenon of designer benzodiazepines and summarizes the available data on pharmacokinetics and pharmacodynamics as well as analytical approaches for their detection.

Keywords
Analysis · Designer benzodiazepines · Metabolism · NPS · Pharmacokinetics · Toxicology

1 Introduction: History and Development of Classic Benzodiazepines

The history of benzodiazepines dates back to the mid-1950s, when the pharmaceutical company Roche started a program aiming at the development of new tranquilizers. Leo Sternbach and his team decided to take an empirical approach by synthesis of benzheptoxdiazines. During the development the chemists noted that they had synthesized quinazoline 3-oxides instead of the desired benzheptoxdiazines (Sternbach 1979). Subsequently, the structure of the new compound was determined as a 1,4 benzodiazepine. In 1958, a patent application was filed, and another year later a patent was granted for various 2-amino-1,4-benzodiazepine 4-oxides. The compound detected in 1957, 7-chloro-2-(methylamino)-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide (generic name: chlordiazepoxide), entered the market in 1960 under the trademark Librium®. Due to their relatively low toxicity, they quickly replaced barbiturates that were regularly used to treat anxiety and insomnia that time. Around 60 years later, benzodiazepines are omnipresent in clinical and forensic laboratories as they are widely prescribed for various psychiatric disorders and have become indispensable medications in anesthesiology and emergency care. On the other hand, the misuse and dependence associated with benzodiazepines worldwide have become a matter of concern.

1.1 Classification

The compounds that are commonly referred to as "benzodiazepines" can be divided in several classes, which are either based on their chemical structure or on their duration of action/elimination half-life:

1.1.1 Chemical Classification

- *1,4-Benzodiazepines*
- 1,5-Benzodiazepines
- Imidazo-benzodiazepines
- *Thienodiazepines*
- *Thienotriazolodiazepines*
- *Triazolobenzodiazepines*
- Oxazolobenzodiazepines

So far, the appearance of only four of these groups (italicized) has been reported in the literature and notified to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). The chemical structures of "designer benzodiazepines" offered via the Internet and of some marketed drugs as examples for the remaining chemical classes are shown in Fig. 1.

1.1.2 Duration of Action

Since many of the benzodiazepines produce active metabolites with sometimes longer elimination half-lives than the parent compound, duration of action cannot be reliably estimated based on the half-life of the ingested drug (see also Sect. 2.6). Additionally, it has to be kept in mind that after redistribution, large amounts of the compound are stored in peripheral tissues and not available at the receptor, thus further limiting the significance of the terminal elimination half-life. Most frequently, the compounds are categorized as follows:

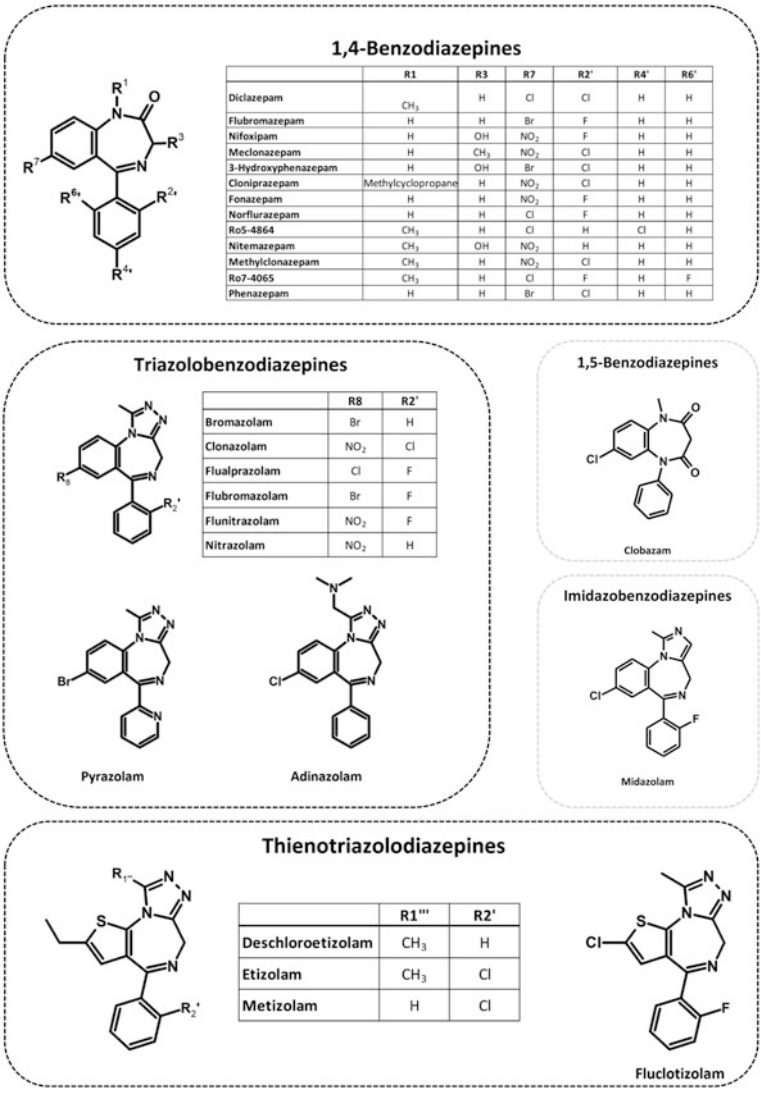
- Short-acting (approx. 1–12 h (e.g., midazolam and triazolam))
- Intermediate-acting (approx. 12–40 h (e.g., oxazepam))
- Long-acting (approx. >40 h (e.g., clonazepam, diazepam))

or

- Short half-life (approx. 1–24 h (e.g., midazolam and triazolam))
- Medium half-life (approx. 24–48 h (e.g., oxazepam))
- Long half-life (approx. >48 h (e.g., clonazepam, diazepam))

1.2 Accumulation

Benzodiazepines having a medium or particularly a long elimination half-life tend to accumulate in various tissues of the human body when ingested repeatedly. Under



	R1	R3	R7	R2'	R4'	R6'
Diclazepam	CH_3	H	Cl	Cl	H	H
Flubromazepam	H	H	Br	F	H	H
Nifoxipam	H	OH	NO_2	F	H	H
Meclonazepam	H	CH_3	NO_2	Cl	H	H
3-Hydroxyphenazepam	H	OH	Br	Cl	H	H
Cloniprazepam	Methylcyclopropane	H	NO_2	Cl	H	H
Fonazepam	H	H	NO_2	F	H	H
Norflurazepam	H	H	Cl	F	H	H
Ro5-4864	CH_3	H	Cl	H	Cl	H
Nitemazepam	CH_3	OH	NO_2	H	H	H
Methylclonazepam	CH_3	H	NO_2	Cl	H	H
Ro7-4065	CH_3	H	Cl	F	H	F
Phenazepam	H	H	Br	Cl	H	H

	R8	R2'
Bromazolam	Br	H
Clonazolam	NO_2	Cl
Flualprazolam	Cl	F
Flubromazolam	Br	F
Flunitrazolam	NO_2	F
Nitrazolam	NO_2	H

	R1'''	R2'
Deschloroetizolam	CH_3	H
Etizolam	CH_3	Cl
Metizolam	H	Cl

Fig. 1 Chemical structures of various designer benzodiazepines detected on the NPS market and some marketed drugs as examples for the remaining chemical classes

certain treatment conditions, for example, anxiety disorders, this can be of advantage since steady-state blood concentrations in the effective range can be reached and maintained with relatively low daily doses. However, for the treatment of sleeping disorders, long elimination half-lives and accumulation are a major disadvantage.

1.3 Indications

Two of the main indications for prescribing benzodiazepines are anxiety and sleep disorders. Additionally, they are utilized to manage withdrawal symptoms from other drugs (e.g., opiates/opioids) and alcohol, in the treatment of seizures (e.g., status epilepticus) and muscle spasms, and as premedication prior to surgery or for analgosedation in intensive care medicine. As far as nonmedical (designer) benzodiazepines are concerned, clinical indications do not exist, and reasons for their popularity and consumption remain speculative. Plausible reasons might include substitution of prescribed benzodiazepines, self-treatment of anxiety or sleep disorders, or use as a standby medication to counteract unpleasant effects induced by other drugs (e.g., stimulants and hallucinogens) (Moosmann et al. 2015).

2 Emergence of Designer Benzodiazepines

Phenazepam (Феназепам), a benzodiazepine originally marketed, e.g., in Russia, was the first benzodiazepine illicitly traded via online "legal high" trading platforms and soon after followed by etizolam, a thienodiazepine marketed under brand names like Etilaam® or Etizest® in a few countries (e.g., India, Japan, and Italy). At the same time, the authentic products containing etizolam were still available. From 2008 to 2011, a growing number of countries with a high misuse and abuse of benzodiazepines in their population (e.g., Sweden, Norway, and Finland) began scheduling phenazepam and later etizolam under national narcotic drug legislation. Consequently, vendors on the illicit drug market turned their attention to other benzodiazepines not regulated on either national or international level. Eventually, phenazepam was added to the Convention on Psychotropic Substance of 1971 (Schedule IV) in March 2016 (UNODC 2018), and etizolam has been recommended for further surveillance since December 2017. Although not being "designer" benzodiazepines in the strict sense due to their availability as medicines in some countries, etizolam and phenazepam are also mentioned in this chapter as these two compounds marked the starting point of the phenomenon and because they are also monitored by the EMCDDA as "new psychoactive substances" (NPS). A more comprehensive summary of both compounds can be found in the critical reviews published by the United Nations Office on Drugs and Crime (UNODC) (UNODC 2018).

With the appearance of pyrazolam (8-bromo-1-methyl-6-(pyridin-2-yl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine) in online shops in mid-2012, the first compound offered on a large scale which was not licensed in any part of the world and not covered by name in any national narcotic law was available. From this perspective, pyrazolam is the first representative of a new class of designer drugs often referred to as "designer benzodiazepines" in analogy to the term designer drugs, which is based on the idea that slight intentional modifications (in the case of pyrazolam, the substitution of a phenyl moiety found in alprazolam with a pyridinyl moiety and the chlorine moeity with a bromine) of an established licit or illicit drug circumvent national laws or international control. The EMCDDA used the term "novel benzodiazepines" in the 2017 drug report (EMCDDA 2017). However, this

might falsely imply that these compounds were synthesized for the first time and neglects the fact that almost all of the offered compounds have been described in either patent or scientific literature. Other names found in recent publications include "new research benzodiazepines" (Wohlfarth et al. 2017) and "new benzodiazepines" (Waters et al. 2017).

Following the emergence of pyrazolam, flubromazepam (7-bromo-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one) and shortly thereafter diclazepam (7-chloro-5-(2-chlorophenyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one) were offered in 2013. Since then, an increasing number of different compounds were offered each year (Table 1).

Designer benzodiazepines have also been detected in fake medicines, e.g., flubromazolam in Xanax® tablets instead of alprazolam (EMCDDA 2018) or the benzodiazepine phenazepam in Erimin 5 tablets instead of nimetazepam in Malaysia and Singapore (Lim et al. 2017).

2.1 Approaches to Drug Development

In terms of "drug design," manufactures seem to choose between four different approaches:

1. Selection of 1,4-benzodiazepines from the scientific literature or patent applications (e.g., from Archer and Sternbach 1968; Sternbach et al. 1962a, b) or logical combinations of substituents based on known structure-activity relationships (see section below) as far as they have not been licensed as medications. It has been suggested that about 3,000 1,4-benzodiazepines have been synthesized in laboratories around the world (Golovenko and Larionov 2014) and compounds contained in the first category include diclazepam (Ro5-3448), Ro5-4864, and Ro7-4065. Flubromazepam is an example for a logical combination of substituents.
2. Selection of active metabolites of known benzodiazepines. Since many benzodiazepines produce pharmacologically active metabolites, the synthesis of these metabolites provides convenient access to new compounds. This approach was also used by the pharmaceutical industry, e.g., by marketing the diazepam metabolites temazepam, nordazepam, and oxazepam. Designer benzodiazepines within this category include norflurazepam, 3-hydroxyphenazepam, fonazepam (norflunitrazepam), nifoxipam (3-hydroxynorflunitrazepam), and nitemazepam.
3. Various modifications of the thienodiazepine etizolam with examples being metizolam, deschloroetizolam, and fluclotizolam.
4. Another feasible way for the production of new compounds is the synthesis of triazolo analogs of available 1,4-benzodiazepines. Since most of the triazolobenzodiazepines show significantly higher potencies, the number of doses from a given amount can be drastically increased with relatively low synthesis effort (Fig. 2) (Hester and Von Voigtlander 1979). Compounds included in this category are, e.g., clonazolam, flubromazolam, flunitrazolam, nitrazolam, and pyrazolam.

Table 1 List of designer benzodiazepines which have been detected on the drug market

Date	Compound	Chemical classification	Systematic name
2012	Pyrazolam	Triazolobenzodiazepine	8-Bromo-1-methyl-6-(pyridin-2-yl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
2013	Diclazepam (Ro5-3448)	1,4-Benzodiazepine	7-Chloro-5-(2-chlorophenyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
	Flubromazepam	1,4-Benzodiazepine	7-Bromo-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
2014	Clonazolam	Triazolobenzodiazepine	6-(2-Chlorophenyl)-1-methyl-8-nitro-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
	Flubromazolam	Triazolobenzodiazepine	8-Bromo-6-(2-fluorophenyl)-1-methyl-4*H*-[1,2,4]triazolo-[4,3-*a*][1,4]benzodiazepine
	Nifoxipam	1,4-Benzodiazepine	5-(2-Fluorophenyl)-3-hydroxy-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
	Meclonazepam	1,4-Benzodiazepine	(3*S*)-5-(2-Chlorophenyl)-3-methyl-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
	Deschloroetizolam	Thienotriazolodiazepine	2-Ethyl-9-methyl-4-phenyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-*a*][1,4]diazepine
2015	3-Hydroxy-phenazepam	1,4-Benzodiazepine	7-Bromo-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
	Metizolam	Thienotriazolodiazepine	4-(2-Chlorophenyl)-2-ethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-*a*][1,4]diazepine
	Nitrazolam	Triazolobenzodiazepine	1-Methyl-8-nitro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine
	Cloniprazepam	1,4-Benzodiazepine	5-(2-Chlorophenyl)-1-(cyclopropylmethyl)-7-nitro-1,3-dihydro-2*H*-[1,4]-benzodiazepin-2-one
	Adinazolam	Triazolobenzodiazepine	1-(8-Chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,5-*a*][1,4]benzodiazepin-1-yl)-*N*,*N*-dimethylmethanamine
2016	Flunitrazolam	Triazolobenzodiazepine	6-(2-Fluorophenyl)-1-methyl-8-nitro-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
	Fonazepam	1,4-Benzodiazepine	5-(2-Fluorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepin-2-one
	Norflurazepam	1,4-Benzodiazepine	7-Chloro-5-(2-fluorophenyl)-1,3-dihydro-1,4-benzodiazepin-2-one

(continued)

Table 1 (continued)

Date	Compound	Chemical classification	Systematic name
	Bromazolam	Triazolobenzodiazepine	8-Bromo-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
	Ro5-4864 (4′-Chlorodiazepam)	1,4-Benzodiazepine	7-Chloro-5-(4-chlorophenyl)-1-methyl-3*H*-1,4-benzodiazepin-2-one
	Nitemazepam	1,4-Benzodiazepine	3-Hydroxy-1-methyl-7-nitro-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one
2017	Flualprazolam	Triazolobenzodiazepine	8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-benzo[f][1,2,4]triazolo[4,3-*a*][1,4]diazepine
	Fluclotizolam	Thienotriazolodiazepine	2-Chloro-4-(2-fluorophenyl)-9-methyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-*a*][1,4]diazepine
	Methylclonazepam	1,4-Benzodiazepine	5-(2-Chlorophenyl)-1-methyl-7-nitro-3*H*-1,4-benzodiazepin-2-one
	Ro7-4065	1,4-Benzodiazepine	7-Chloro-5-(2,6-difluorophenyl)-1-methyl-3*H*-1,4-benzodiazepin-2-one
	Thionordazepam	1,4-Benzodiazepine	7-Chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-thione

Systematic names (IUPAC) and year of first occurrence are given

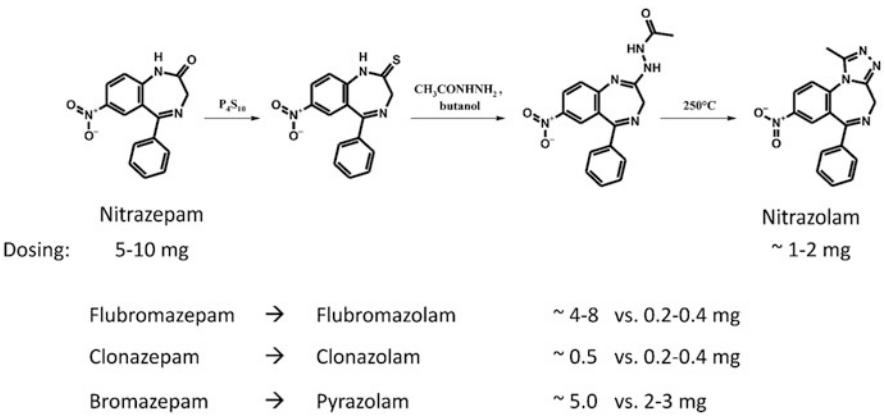


Fig. 2 Simplified synthetic route for the transformation of 1,4-benzodiazepines to triazolobenzodiazepines (Kleemann et al. 2014) along with examples for the reduction of tentative doses due to increase of potency (Hester et al. 1971)

2.2 Pharmacology

2.2.1 $GABA_A$ Receptor

The pharmacological target of benzodiazepines is the central γ-aminobutyric acid type A ($GABA_A$) receptor located in post- and presynaptic membranes (Mohsin and Qadir 2015). $GABA_A$ receptors are ligand-gated ion channels, and activation leads to hyperpolarization and inhibition of neurotransmission. The endogenous ligand of the receptor is γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (Waters et al. 2017). $GABA_A$ receptors are composed of five heteromeric protein subunits and are abundantly expressed in organisms with a nervous system (Olsen and Sieghart 2008). At least 16 human $GABA_A$ receptors have been described with 7 distinct subfamilies of subunits (α, β, γ, ρ, δ, ε, and θ), whereby most of them are made up of two α-, two β-, and one γ-subunit. In contrast to agonists (e.g., muscimol), benzodiazepines bind as positive allosteric modulators at the $GABA_A$ receptor. Therefore, they do not directly activate this receptor but enhance the action of GABA by increasing the frequency of GABA-activated channel opening and by increasing channel conductance (Chebib and Johnston 2000). This mechanism is the basis of the broad therapeutic range of benzodiazepines in contrast to barbiturates where the maximum effect is not self-limited. The effects are completely dependent on the subunit composition (Barnard et al. 1987). The binding site of benzodiazepines is located at the interface of an α- and a γ-subunit, with receptors carrying the γ_2-subtype being more sensitive than the ones with the γ_1-subtype (Olsen and Sieghart 2008). Depending on the α-subtype, benzodiazepines can show different affinity, with, e.g., some $GABA_A$ receptors containing α_4- or α_6-subunits with β and γ_2 not binding traditional benzodiazepines (Archer and Sternbach 1968).

Based on studies with genetically engineered mice, it was demonstrated that the different receptor subtypes mediate the different effects observed after benzodiazepine intake. The $\alpha_1\beta\gamma_2$ receptors mediate the sedative, the anterograde amnestic, and in part the anticonvulsant effects of diazepam, whereas the $\alpha_2\beta\gamma_2$ receptors mediate the anxiolytic and muscle relaxant activity (Mohsin and Qadir 2015). The benzodiazepine analogs zolpidem and zaleplon (so-called z-drugs) bind to the $\alpha_1\beta\gamma_2$ receptors while lacking pronounced affinity to the $\alpha_2\beta\gamma_2$ receptors, thus showing less anxiolytic and muscle relaxant activity (Bönisch 2007).

So far, there are no data published on the subtype specificity of designer benzodiazepines. Consequently, the effects of newly emerging compounds cannot be predicted with certainty. Nevertheless, there is a high probability that effects in humans are similar to medically used benzodiazepines and that the effects will most probably be antagonized by flumazenil. Etizolam was shown to possess more anxiolytic than sedative properties, probably due to its lower intrinsic activity at $GABA_A$ receptors containing an α_1-subunit (Sanna et al. 1999).

One compound marking an exception in terms of typical benzodiazepine-like effects is 4′-chlorodiazepam (Ro5-4864). This compound has been studied extensively because of its binding to the translocator protein (TSPO), also known as the peripheral benzodiazepine receptor. Ro5-4864 was reported to possess proconflict

and anxiogenic effects as well as convulsant action. However, Ro5-4864 shows distinct differences in binding among various species (Gavish et al. 1999).

2.2.2 Effects

All medically used benzodiazepines and almost all designer benzodiazepines offered so far mediate very similar effects (an exception is Ro5-4864; see above). They have:

- Anxiolytic
- Muscle relaxant
- Sedative, amnestic, and hypnotic
- Anticonvulsive

activity

A slight regulation of the desired effects can be achieved by adjusting the drug dose; at low doses the anxiolytic effects are triggered, and with increasing doses, the sedative, amnestic, muscle-relaxing, hypnotic, and lastly anticonvulsive effects appear (Bönisch 2007).

As the major effects of most benzodiazepines do not differ, the choice in clinical settings is usually based on the differences in elimination half-lives and potencies of the compounds. Nevertheless, there might be differences between designer benzodiazepines regarding, e.g., the amnestic potential with implications for a potential use of these drugs for drug-facilitated crimes.

In vivo data on the effects of designer benzodiazepines in the literature are scarce. Based on scientific self-experiments by one volunteer, doses of 1 mg pyrazolam and 1 mg diclazepam on separate occasions were reported to cause no noticeable effects (Moosmann et al. 2013b, 2014). A dose of 4 mg flubromazepam was reported by the same volunteer to have caused "some fatigue and an enhanced need of sleep for three consecutive days" (Moosmann et al. 2013a). The effects of a dose of 0.5 mg of flubromazolam was described by Huppertz et al. (2018) as follows: "Approximately 90 min after the drug intake, the volunteer noticed muscle-relaxing effects and onset of a light tiredness. The volunteer seemed visibly impaired and experienced strong sedative effects and repeatedly fell asleep starting from 3 h post drug ingestion, lasting for more than 10 h. He had difficulties following or participating in conversations. Additionally, he developed partial amnesia enduring far over 24 hours . . ." (Huppertz et al. 2018). Another self-experiment with a dose of 2 mg metizolam was reported to cause no noticeable effects by Kintz et al. (2017b). Data found on the Internet regarding "common dosing" of designer benzodiazepines are listed in Table 2 (Tripsit 2018). It goes without saying that the depicted data can only serve as a first approximation until more reliable, scientific data become available since it has been shown that benzodiazepines with very similar chemical structure can have significantly different pharmacological properties, for example, in terms of potency and rate of absorption (Chouinard 2004).

Table 2 Tentative doses of designer benzodiazepines (Tripsit 2018) along with their predicted binding value (Waters et al. 2017), mean experimental log $D_{7.4}$, and pK_a values (pK_a1, deprotonation of the nitrogen atom at position 4; pK_a2, deprotonation of the nitrogen atom at position 1) as well as its mean plasma protein binding (PPB) (Manchester et al. 2018)

	"Common dose"[a] [mg]	Predicted binding value (log 1/c)	Log $D_{7.4}$	pK_a1	pK_a2	PPB
Flunitrazolam	0.08–0.15	8.88				
Clonazolam	0.2–0.4	8.86				
Flubromazolam	0.2–0.4	8.77	2.40	2.07	None	89.5
Etizolam	1–2	8.64	2.40	2.83	None	92.8
Nifoxipam	0.5–1	8.63				
Meclonazepam	3–6	8.52	2.64	2.10	11.45	88.2
Fonazepam	1–2	8.46				
Norflurazepam	5–10	8.44	2.82	2.51	11.64	95.5
3-OH-Phenazepam	1–2	8.42	2.54	1.25	11.96	97.7
Diclazepam	2–3	8.39	2.73	2.31	None	93.8
Flubromazepam	4–8	8.37	2.87	3.25	10.74	96.4
Metizolam	2–4	8.34				
Nitrazolam	1–2	8.34				
Bromazolam	1–3	8.25				
Phenazepam	1–2	8.12	3.25	2.19	11.21	98.3
Deschloroetizolam	4–6	7.96	2.60	4.19	None	87.2
Ro5-4864		7.88	2.75	3.13	None	98.2
Nitemazepam	1.5–3	7.87				
Cloniprazepam	1–2	7.83				
Pyrazolam	2–3	7.79	0.97	3.30	None	78.7
Adinazolam	15–30	7.18				
Flualprazolam	0.25–0.5	n.t.				
Fluclotizolam	0.25–0.5	n.t.				

n.t. not tested
[a]According to Tripsit (2018)

2.2.3 Pharmacological Activity of Metabolites

Phase I metabolites of benzodiazepines are commonly pharmacologically active although exceptions exist, such as the amino metabolites of benzodiazepines carrying a nitro moiety (e.g., the 7-amino metabolites of nitrazepam and clonazepam). Therefore, some metabolites have also been marketed as medicines in their own right, such as the main metabolites of diazepam (temazepam, nordazepam, and oxazepam). In the case of alprazolam and triazolam, the α-hydroxy metabolites retain a high binding affinity with α-hydroxytriazolam being at least as active as the parent compound (Hester and Von Voigtlander 1979). In contrast, the 4-hydroxy metabolites commonly show reduced activity (Baselt 2011). For midazolam, Bauer et al. showed that α-hydroxymidazolam glucuronide, a phase II metabolite, also

retains significant binding affinity at the benzodiazepine binding site of the receptor (Bauer et al. 1995).

2.3 SAR and Drug Design

Based on the early work of Sternbach et al. and Hester et al., the following structure-activity relationships of 1,4-benzodiazepines and triazolobenzodiazepines were observed (Fig. 3) (Hester and Von Voigtlander 1979; Sternbach et al. 1968).

The substituent on C-7 (C-8 for triazolobenzodiazepines) is of paramount importance (Sternbach 1971). The activity can be increased by an electron-withdrawing substituent (e.g., halogen, CF_3, or NO_2). In the case of 1,4-benzodiazepines, an increase in activity was observed alongside an increase in electronegativity, although this was not observed for triazolobenzodiazepines. For both groups of structures, a halogen (F or Cl) present in the *ortho*-position of the phenyl ring leads to a significantly enhanced activity. For 1,4-benzodiazepines, substitution at *N*-1 has a decisive effect on the activity, and for triazolobenzodiazepines, a methyl substituent at C-1 showed the most significant increase in activity (tested H to n-propyl), and bulky substituents such as a phenyl groups led to loss of activity. For 1,4-benzodiazepines, a decrease in activity has been described with an electron donor present at C-7 and a substituent in the *para*-position of the phenyl ring. Substitutions at C-6, C-8, and C-9 also lowered the activity of the compounds (Sternbach et al. 1968). Furthermore, a substituent at C-3 generally resulted in a decrease in potency and the phenyl ring at C-5 appeared to be the best option with

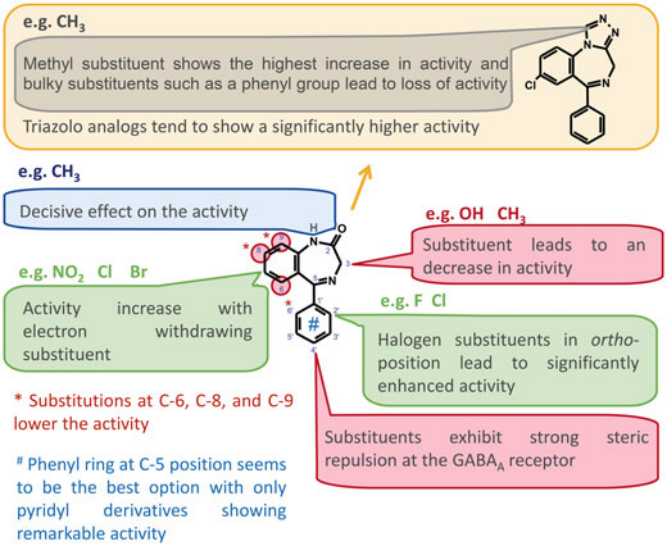


Fig. 3 Summary of structure-activity relationships of benzodiazepines described in the literature

only pyridyl derivatives showing remarkable activity. Substituents on the R4′ position of the phenyl ring are known to exhibit strong steric repulsion at the $GABA_A$ receptor (Waters et al. 2017). Substitution of the carbonyl function at C-2 by a thione leads to considerably lower activity (Sternbach et al. 1968). It can therefore be assumed that the emergence of, e.g., thionordazepam is aimed at providing a precursor for the synthesis of alprazolam (see Fig. 2) (Hester and Von Voigtlander 1979) rather than being sold as a drug itself.

Waters et al. utilized a quantitative structure-activity (QSAR) approach to predict the binding affinities of designer benzodiazepines becoming available on the drug market (Waters et al. 2017). For this purpose, a model was created based on 69 known benzodiazepines, which was applied to predict the binding affinities of 22 designer benzodiazepines. In their model important binding features of the benzodiazepines were the position of two H-bond acceptors, two aromatic rings, and a hydrophobic group. Based on the data generated with the QSAR model for the 22 tested compounds, these benzodiazepines in general showed a greater binding affinity toward the $GABA_A$ receptor than most of the "classic" benzodiazepines available as prescription medicines. The three compounds with the highest predicted binding affinities (log 1/c) were all triazolobenzodiazepines (flunitrazolam, clonazolam, and flubromazolam). A summary of the data generated with the model of Waters et al. can be found in Table 2 along with common doses reported by drug users.

2.4 Metabolism

Most of the designer benzodiazepines get extensively metabolized prior to excretion. The metabolic steps in general resemble the reactions described in the literature for "classic" benzodiazepines (UNODC 1997).

For an overview, general metabolic steps for certain subclasses of benzodiazepines are depicted in Fig. 4. In the following part, in vivo and in vitro data for specific compounds are given along with pitfalls regarding the interpretation of metabolite findings. With the exception of adinazolam (Fraser et al. 1993; Lahti et al. 1983), phenazepam (Maskell et al. 2012; Zherdev et al. 1982) and etizolam (Fracasso et al. 1991; Nakamae et al. 2008), all of the metabolic data published were assessed as a reaction to the emergence of the compound on the "legal high" drug market.

Phase I:

- Hydroxylation

  1,4-Benzodiazepines: mainly at the C-3 position, minor site is C-4′ (Breimer 1979).

  Imidazo- and triazolo-compounds: mainly at the methyl group of the annealed heterocycle or at the C-4 position (Breimer 1979; Gorski et al. 1999; Kitagawa et al. 1979).

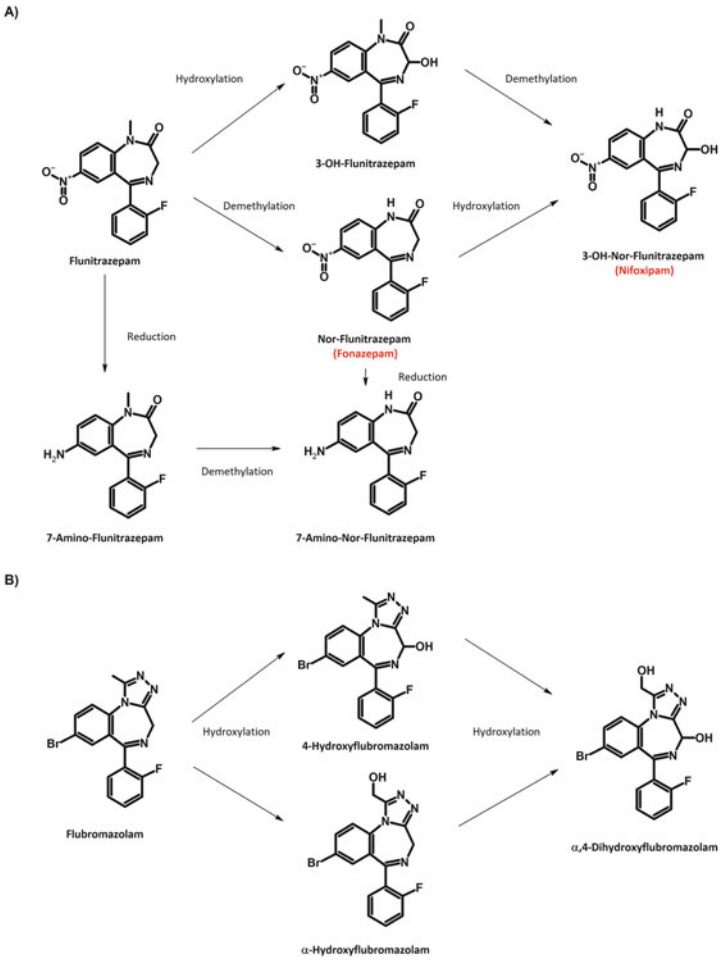


Fig. 4 Metabolic steps. (**a**) Phase I metabolism of flunitrazepam as an example for the common metabolic steps observed with 1,4-benzodiazepines and as an example for benzodiazepines carrying a nitro moiety. (**b**) Phase I metabolism of flubromazolam as an example for the common metabolic steps observed with triazolobenzodiazepines

- Dealkylation

  1,4-Benzodiazepines carrying an alkyl moiety at *N*-1 are often prone to dealkylation (Schwartz et al. 1965).
- Reduction of nitro moiety

  The nitro moiety of 1,4-benzodiazepines (at the C-7 position) or triazolobenzodiazepines (at the C-8 position) are reduced to the respective amine (Coller et al. 1999; Mattila and Larni 1980).

Phase II:

- *O*-Glucuronidation
  In urine, benzodiazepines carrying a hydroxyl moiety (or the hydroxylated metabolites of benzodiazepines) are excreted as conjugates of glucuronic acid. In the case of, e.g., lorazepam and oxazepam, glucuronidation can be almost complete (Greenblatt 1981; Hyland et al. 2009).
- *N*-Glucuronidation
  The imidazo-benzodiazepine midazolam and triazolobenzodiazepines are partly excreted as *N*-glucuronides of the parent compound. In the case of midazolam, the conjugation occurs at the *N*-2 position (Hyland et al. 2009).
- Acetylation
  The amine function of reduced nitro (triazolo-) benzodiazepines can be acetylated metabolically.

2.4.1 Pyrazolam

The first study on the metabolism of pyrazolam, the first designer benzodiazepine on the market, was published in 2013. At the time, no metabolite was detected after the intake of 1 mg by one volunteer, and the unchanged parent compounds could be detected in urine in concentrations reaching up to 160 ng/mg normalized to a creatinine concentration of 100 mg/dL (Moosmann et al. 2013b). The authors hypothesized that pyrazolam was not metabolized extensively prior to excretion, and this hypothesis was later supported by Pettersson Bergstrand et al. who also identified the parent compound as the main analytical target in urine. However, the authors of the second study detected three metabolites in samples obtained from 13 authentic case samples. Pyrazolam glucuronide was detected in all samples at presumably low concentrations ($n = 13$) and two hydroxy metabolites also showed low abundance (Pettersson Bergstrand et al. 2017b).

2.4.2 Flubromazepam

First in vivo data on the metabolism of flubromazepam were published in 2013. After the intake of 4 mg of the compound by a human volunteer, two hydroxy metabolites, a debrominated and a debrominated/hydroxylated metabolite were identified (Moosmann et al. 2013a). The main hydroxyl metabolite was later identified as 3-hydroxyflubromazepam using certified reference material (own unpublished data). In urine, the hydroxy metabolites were predominantly present as conjugates with glucuronic acid, and only small amounts of unmetabolized flubromazepam were detected. The formation of debrominated metabolites was rather surprising and might be explained by bacterial degradation of flubromazepam in the gastrointestinal system. Presence of debrominated flubromazepam metabolites was also reported later by Rudolph et al. (2015).

2.4.3 Diclazepam

Diclazepam undergoes the same metabolic reactions as the closely related diazepam. After uptake of 1 mg of the compound, the demethylated (delorazepam), hydroxylated (lormetazepam), and demethylated/hydroxylated (lorazepam)

metabolites could be detected. In the analyzed urine samples, all three metabolites were conjugated to glucuronic acid, suggesting that delorazepam was excreted in the form of the *N*-glucuronide. Similar to diazepam, diclazepam was only detected in traces in urine samples (Moosmann et al. 2014).

2.4.4 Flubromazolam

One of the best-studied designer benzodiazepines so far is flubromazolam. A number of publications regarding in vitro and in vivo metabolism data are available (El Balkhi et al. 2017; Huppertz et al. 2015, 2018; Noble et al. 2017; Pettersson Bergstrand et al. 2017b; Wohlfarth et al. 2017). Wohlfarth et al. (2017) detected a total of seven metabolites for this compound in six authentic urine samples. The predominant biotransformation reactions were hydroxylation, dihydroxylation, and *O*-glucuronidation. Additionally, an *N*-glucuronide was detected. In analogy to midazolam (Noble et al. 2017), the predominant metabolite observed in these samples was α-hydroxyflubromazolam. The in vivo results reported by Pettersson Bergstrand et al., which were based on the analysis of 22 authentic urine samples, of Noble et al. (2017) and Huppertz et al. (2018), were comparable and added two further glucuronides. The authors also observed that the concentration of the parent compound increased about 2–19-fold (mean about 9-fold) after hydrolysis of the urine samples, indicating the parent compound being excreted to a high extent in its glucuronidated form.

2.4.5 Clonazolam

So far, seven in vivo metabolites of clonazolam have been identified in human urine. The main metabolites described were 8-aminoclonazolam and 8-acetamidoclonazolam and one monohydroxylated metabolite (probably α-position). Two monohydroxylated and glucuronidated metabolites as well as the glucuronides of 8-aminoclonazolam and 8-acetamidoclonazolam were detected (Meyer et al. 2016). The parent compound was detected in relatively low concentrations in all authentic urine samples ($n = 4$).

2.4.6 Nifoxipam

For nifoxipam, the parent compound was not detected in authentic urine samples ($n = 4$) (Meyer et al. 2016). This might in part be explained by the observation that nifoxipam is not stable in biological samples even when stored at −20 °C (own unpublished data). Additionally, the sensitivity of mass spectrometer detectors for this compound was found to be low in a variety of instruments equipped with electrospray ionization (ESI) sources (own unpublished data). The only three metabolites identified in vivo were nifoxipam glucuronide (proposed main metabolite in urine), 7-aminonifoxipam, and 7-acetaminonifoxipam (Meyer et al. 2016).

2.4.7 Meclonazepam

Two studies regarding the in vivo metabolism of meclonazepam have been published (Meyer et al. 2016; Vikingsson et al. 2017). Both studies were based on the analysis of authentic urine samples and report the identification of the same main metabolites 7-aminomeclonazepam and 7-acetaminomeclonazepam. In addition, the

parent compound was detected in all investigated urine samples ($n = 7$) by Meyer et al. and in 3 of the 4 analyzed urine samples by Vikingsson et al., who detected an additional 11 metabolites in human urine at relatively low abundances.

2.4.8 Metizolam

First data on the in vivo metabolism of metizolam was published by Kintz et al. who analyzed urine samples after the intake of 2 mg by a volunteer. Metizolam was excreted at low concentrations in urine (maximum about 10 ng/mL), and 35-h post-intake only 0.3% of the dose was excreted unchanged via urine. The main metabolites identified were one monohydroxylated metabolite, its glucuronide, and low amounts of a second monohydroxylated metabolite. The position of the hydroxylation of the metabolites was not investigated by the authors (Kintz et al. 2017b). A summary of all data published on in vivo and in vitro metabolism so far is given in Table 3.

2.5 Metabolizing Enzymes

Very few data on the enzymes involved in the metabolism of designer benzodiazepines have been published. Noble et al. investigated the metabolism using recombinant cytochrome P450 (CYP) enzymes and attributed formation of α-hydroxyflubromazolam, 4-hydroxyflubromazolam, and α,4-dihydroxyflubromazolam to CYP3A4 and 3A5 (Noble et al. 2017). These data were in agreement with the studies carried out using other triazolo-/imidazo-benzodiazepines like alprazolam, triazolam, and midazolam where enzymes of the CYP3A family have been shown to be involved in the hydroxylation at the α- and 4-position (Masica et al. 2004). Similarly, CYP3A4 and CYP2C19 are principal enzymes involved in the phase I metabolism of, e.g., diazepam and flunitrazepam (Kilicarslan et al. 2001).

Based on their metabolism studies with human liver microsomes under anaerobic conditions Vikingsson et al. suggested the reduction of the nitro moiety in meclonazepam to be catalyzed by CYP3A4 and the conjugation leading to 7-acetamidomeclonazepam by NAT2 (Vikingsson et al. 2017).

2.6 Elimination Half-Life

The most relevant differences between various benzodiazepines are their elimination half-lives. Repeated intake of long-acting benzodiazepines inevitably leads to bioaccumulation, providing the possibility of analytical detection for a long time period after cessation of use. On the other hand, the risk of intoxication and adverse effects rises if the consumer takes the next doses too early. Short-acting, potent benzodiazepines (e.g., triazolam) might go undetected following a single intake, for example, in the context of drug-facilitated crimes (DFC).

Table 3 Literature data on the phase I metabolism of designer benzodiazepines

Compound	Anticipated metabolic steps (phase I)	In vitro data	In vivo phase I data	Reference
3-HO-Phenazepam	None	None detected	n/a	Moosmann et al. (2016)
Adinazolam	Dealkylation	*N*-Demethyladinazolam *N*,*N*-Didemethyladinazolam	*N*-Demethyladinazolam *N*,*N*-Didemethyladinazolam α-Hydroxy-alprazolam Estazolam	Lahti et al. (1983)
Bromazolam	Hydroxylation	n/a	n/a	
Clonazolam	Reduction Hydroxylation	8-Aminoclonazolam Hydroxyclonazolam	8-Aminoclonazolam 8-Acetamidoclonazolam Hydroxyclonazolam	El Balkhi et al. (2017), Huppertz et al. (2015), and Meyer et al. (2016)
Cloniprazepam	Dealkylation Reduction Hydroxylation	Hydroxycloniprazepam Dihydroxycloniprazepam 7-Aminocloniprazepam Ketocloniprazepam Clonazepam (dealkylcloniprazepam) 7-Aminoclonazepam 3-Hydroxy-7-aminoclonazepam Two hydroxyclonazepam metabolites (presumably at position 3 and 4′)	n/a	Moosmann et al. (2016) and Mortelé et al. (2018)
Norflurazepam	Hydroxylation	Two hydroxynorflurazepam metabolites (presumably at position 3 and 4′) Dihydroxynorflurazepam	n/a	Own unpublished data
Deschloroetizolam	Hydroxylation	Hydroxydeschloroetizolam (suggested 9-methyl position) Hydroxydeschloroetizolam (suggested 2-ethyl position) Hydroxydeschloroetizolam (suggested C-6-position) Dihydroxydeschloroetizolam	n/a	El Balkhi et al. (2017) and Huppertz et al. (2015)

Diclazepam	Dealkylation Hydroxylation	Lormetazepam Delorazepam Lorazepam	Lormetazepam Delorazepam Lorazepam	El Balkhi et al. (2017) and Moosmann et al. (2014)
Flualprazolam	Hydroxylation	n/a	n/a	
Flubromazepam	Hydroxylation	3-Hydroxyflubromazepam Debromoflubromazepam Hydroxyflubromazepam	3-Hydroxyflubromazepam Debromoflubromazepam Hydroxyflubromazepam	El Balkhi et al. (2017) and Moosmann et al. (2013a)
Flubromazolam	Hydroxylation	α-Hydroxyflubromazolam 4-Hydroxyflubromazolam Dihydroxyflubromazolam	α-Hydroxyflubromazolam 4-Hydroxyflubromazolam Hydroxyflubromazolam (position unknown) α,4-Dihydroxyflubromazolam	El Balkhi et al. (2017), Huppertz et al. (2018), Noble et al. (2017), Pettersson Bergstrand et al. (2017b), and Wohlfarth et al. (2017)
Fluclotizolam	Hydroxylation	n/a	n/a	
Flunitrazolam	Reduction Hydroxylation	8-Aminoflunitrazolam Hydroxyflunitrazolam (suggested α-position) Hydroxyflunitrazolam (suggested 4-position) Dihydroxyflunitrazolam (suggested α-position and 4-position) 8-Aminohydroxyflunitrazolam (suggested α-position or 4-position)	n/a	Own unpublished data
Fonazepam	Reduction Hydroxylation	7-Aminofonazepam 3-Hydroxyfonazepam (nifoxipam) Hydroxyfonazepam (suggested 4-position)	n/a	Moosmann et al. (2016)
Meclonazepam	Reduction Hydroxylation	7-Aminomeclonazepam 7-Acetaminomeclonazepam Hydroxymeclonazepam	7-Aminomeclonazepam 7-Acetamidomeclonazepam	Huppertz et al. (2015), Meyer et al. (2016), and Vikingsson et al. (2017)

(continued)

Table 3 (continued)

Compound	Anticipated metabolic steps (phase I)	In vitro data	In vivo phase I data	Reference
Methylclonazepam	Dealkylation Reduction Hydroxylation	n/a	n/a	
Metizolam	Hydroxylation	Two hydroxymetizolam metabolites Dihydroxymetizolam	Two hydroxymetizolam metabolites	Kintz et al. (2017b) and Moosmann et al. (2016)
Nifoxipam	Reduction	7-Aminonifoxipam	7-Aminonifoxipam 7-Acetamidonifoxipam	El Balkhi et al. (2017) and Meyer et al. (2016)
Nitemazepam	Reduction Dealkylation	n/a	n/a	
Nitrazolam	Reduction Hydroxylation	Hydroxynitrazolam (suggested α-position or 4-position) 8-Aminonitrazolam	n/a	Moosmann et al. (2016)
Ro5-4864	Dealkylation Hydroxylation	Nor-Ro5-4864 Hydroxy-Ro5-4864 Hydroxy-nor-Ro5-4864	n/a	Own unpublished data
Ro7-4065	Dealkylation Hydroxylation	n/a	n/a	
Pyrazolam	Hydroxylation	Two hydroxypyrazolam metabolites	α-Hydroxypyrazolam 4-Hydroxypyrazolam	Moosmann et al. (2013b) and Pettersson Bergstrand et al. (2017b)

n/a no data available

Table 4 Pharmacokinetic data of designer benzodiazepines and diclazepam metabolites (lorazepam, delorazepam, and lormetazepam)

Compound	Dose ingested [mg]	c_{max} [ng/mL]	Compartment model	Elimination half-life [h]	Reference
Pyrazolam	1	51	1	17	Moosmann et al. (2013b)
Flubromazepam	4	78	1	106	Moosmann et al. (2013a)
Diclazepam	1	3.4	2	1.9 (initial), 42 (terminal)	Moosmann et al. (2014)
Lormetazepam				13	Hümpel et al. (1979)
Delorazepam				78	Bo et al. (1980)
Lorazepam				12	Greenblatt et al. (1976)
Flubromazolam	0.5	8.6	Not stated	10–20	Huppertz et al. (2018)
Norflurazepam				40–100	Chouinard (2004)

Estimated values for elimination half-lives have only been published for few designer benzodiazepines in the literature so far. Most of these values are based on data from one volunteer after the intake of, in most cases, a rather light dose of the drug. The elimination half-life of norflurazepam has been published since it is an active metabolite of the prescription benzodiazepine flurazepam. Additionally, the elimination half-lives of the diclazepam metabolites (lormetazepam, delorazepam, and lorazepam) are known. The (estimated) elimination half-lives known so far are listed in Table 4.

2.7 Further Pharmacokinetic Parameters of Designer Benzodiazepines

2.7.1 Plasma Protein Binding, pK_a, and Log $D_{7.4}$

Plasma protein binding, pK_a, and log $D_{7.4}$ (distribution coefficient) of 11 designer benzodiazepines were determined by Manchester et al. and are listed in Table 2 (Manchester et al. 2018). Plasma protein binding of flubromazolam was also determined by Noble et al. to be 89% ($f_{u,p}$: 0.11) and is therefore ranging between the values for alprazolam and diazepam in the experiments (Noble et al. 2017).

2.7.2 Hepatic Clearance and Volume of Distribution

Based on in vitro experiments, the predicted hepatic clearance of flubromazolam was reported at 0.42 mL/min/kg or 0.43 mL/min/kg depending on the apparatus used, suggesting a rather long elimination half-life (Noble et al. 2017).

2.7.3 Permeability of the Blood-Brain Barrier

Most benzodiazepines, including designer benzodiazepines, are highly lipophilic implying that they readily cross the blood-brain barrier. The ratio of the concentration of phenazepam and 3-hydroxyphenazepam in brain and blood was determined in mice by Golovenko et al. and reported to be around 1.1–1.3 for phenazepam and 0.83–0.93 for 3-hydroxyphenazepam (Golovenko and Larionov 2014).

2.7.4 Concentrations in Biological Samples

Høiseth et al. published concentrations of five designer benzodiazepines (clonazolam, diclazepam, flubromazepam, flubromazolam, and pyrazolam) based on the analysis of 77 authentic forensic blood samples. The range of concentrations were 0.48–100 ng/mL for flubromazolam ($n = 25$), 4.7–1,200 ng/mL for flubromazepam ($n = 24$), 2.1–57 ng/mL for diclazepam ($n = 15$), 1.9–11 ng/mL for clonazolam ($n = 7$), and 7.4 ng/mL for pyrazolam ($n = 1$) (Høiseth et al. 2016). Maximum serum concentrations reached after intake of a defined dose of a designer benzodiazepine are listed in Table 4.

2.8 Intoxications

Łukasik-Głębocka et al. reported a severe intoxication associated with coma, hypotension, and rhabdomyolysis after the intake of a self-stated dose of 3 mg flubromazolam. Improvement of the state of consciousness of the patient occurred on day 4 of treatment in hospital. The authors describe that flumazenil (1.0 mg) improved the patient's consciousness for about 30 min, which suggests that this benzodiazepine antagonist may be effective in the treatment of flubromazolam (and other designer benzodiazepine) intoxications. The flubromazolam serum concentration in this case was 43 ng/mL (Łukasik-Głębocka et al. 2016).

2.9 Methods of Detection

Various methods of detection of designer benzodiazepines in biological matrices have been published. Most designer benzodiazepines can be detected with sufficient sensitivity by immunochemical assays in urine samples (O'Connor et al. 2015; Pettersson Bergstrand et al. 2017a). This is in contrast to other classes of NPS, such as synthetic cannabinoid receptor agonists, for which suitable immunochemical assays are lacking on the market to date (Franz et al. 2017).

2.9.1 Immunochemical Assays

The most comprehensive evaluation of immunochemical assays used for the detection of designer benzodiazepine intake in urine was carried out by Pettersson Bergstrand et al. who evaluated four different benzodiazepine immunoassays (CEDIA, HEIA, EMIT II Plus, and KIMS II) with nine designer benzodiazepines and with estazolam, etizolam, flutazolam, as well as phenazepam. Many of the

compounds tested showed good cross-reactivity across all four tests. On the CEDIA and EMIT II Plus assay, only flutazolam showed poor (3 and 4%) and meclonazepam and nifoxipam low (10–39%) cross-reactivity. Utilizing the HEIA assay, deschloroetizolam, etizolam, and flutazolam did not perform well. The KIMS II performed well with all compounds, with nifoxipam showing the lowest cross-reactivity (37%) (Pettersson Bergstrand et al. 2017a). These data were consistent with results from other studies where designer benzodiazepines also showed sufficient cross-reactivity when tested with immunochemical assays (Huppertz et al. 2018; Moosmann et al. 2013a, b, 2014; O'Connor et al. 2015).

Although these assays perform well in urine due to sufficient cross-reactivity, a few aspects have to be kept in mind:

- Cutoff: "clinical cutoffs" recommended by the assay manufacturer are usually not sufficient for the detection of a one-time intake/application of potent designer benzodiazepines, even if the assay shows sufficient cross-reactivity.
- Application in blood/serum: blood levels of potent designer benzodiazepines can be very low (e.g., flubromazolam well below 10 ng/mL). Consequently, immunochemical assays might not be suitable for screening in certain cases (e.g., DFC).

In general, immunochemical assays perform well, but an important aspect is to keep confirmation methods (e.g. LC-MS/MS) up to date. Otherwise, a positive immunoassay might be erroneously considered a "false positive." Data from Sweden showed that about 40% of the benzodiazepine immunoassay positive urine samples tested negative for prescription benzodiazepines but positive for designer benzodiazepines (Pettersson Bergstrand et al. 2016).

2.9.2 Mass Spectrometric Methods

In the beginning of the designer benzodiazepine phenomenon, methods of detection applied in the context of scientific self-experiments focused on the detection of a particular compound (Kintz et al. 2017b; Moosmann et al. 2013a, b, 2014). By now, a number of multi-analyte methods have been published utilizing either low- or high-resolution instrumentation coupled with liquid chromatography (LC) (Høiseth et al. 2016; Peter et al. 2017; Pettersson Bergstrand et al. 2016; Tomkova et al. 2017), gas chromatography (GC) (Meng et al. 2017), or capillary electrophoresis (CE) systems (Švidrnoch et al. 2018).

2.9.3 Detection in Alternative Matrices

A single intake of flubromazolam (0.5 mg) and metizolam (2 mg) could be detected in hair although resulting in very low concentrations. For flubromazolam, a highly sensitive LC-MS^3 method was applied detecting a maximum of 0.6 pg/mg in hair segments (LOD 0.01 pg/mg) (Huppertz et al. 2018). Metizolam was detected at a concentration of up to 0.27 pg/mg utilizing an LC-MS/MS method and additionally in sweat samples and exhaled breath (Kintz et al. 2017a). A single administration by one volunteer of 0.25 mg flunitrazolam was detected in oral fluid between 1–8 h, and

Table 5 Designer benzodiazepines involving the risk of falsely interpreting analytical findings

Compound	Category	"Critical" metabolites	Risk of false interpretation
3-Hydroxy-phenazepam	[a]		Phenazepam uptake
Adinazolam	[b]	Estazolam α-OH-Alprazolam	Estazolam or alprazolam uptake
Cloniprazepam	[b]	Clonazepam and metabolites thereof	Clonazepam uptake
Diclazepam	[b]	Delorazepam Lormetazepam Lorazepam	Delorazepam and/or lormetazepam and/or lorazepam uptake
Fonazepam	[a]		Flunitrazepam uptake
Nifoxipam	[a]		Flunitrazepam or fonazepam uptake
Norflurazepam	[a/b]	Hydroxynorflurazepam	Flurazepam or fludiazepam uptake Metabolite --> cinolazepam uptake

[a]Metabolite of another marketed benzodiazepine
[b]Metabolized to prescription benzodiazepines

the concentration ranged from 7 to 178 pg/mL, stressing the need for highly sensitive instrumentation for the detection of these drugs (Ameline et al. 2018).

2.9.4 Interpretation of Analytical Findings

Due to the sale of metabolites (e.g., fonazepam and norflurazepam) and of compounds metabolized to "classic" benzodiazepines (e.g., diclazepam and cloniprazepam), interpretation of the analytical findings can be complicated (Table 5).

Ro5-4864 (4′-chlorodiazepam) and its metabolites show fragmentation patterns very similar to the patterns of its positional isomer diclazepam (2′-chlorodiazepam) and its metabolites (delorazepam, lormetazepam, lorazepam). Therefore, sufficient chromatographic separation and verification of ion ratios are inevitable.

3 Conclusions

In the last years, a growing number of designer benzodiazepines appeared on "legal high" markets. In general, the pharmacological profiles of the new compounds seem to be very similar to the profiles reported of known benzodiazepines marketed as medicines. However, there may be additional risks due to missing clinical evaluation and potential, hitherto unknown toxicity. In the forensic and clinical context, highly specific methods for the detection of the new compounds and their metabolites are required to avoid false-negative findings following positive immunochemical tests. Due to the high frequency of appearance of new designer benzodiazepines, their complex metabolism, and the high potency of some of these compounds resulting in very low concentrations in biological matrices, setting up and maintaining comprehensive and reliable analytical methods for their detection can be very challenging. The data presented in this chapter might support the development of analytical

methods as well as clinical and forensic interpretation of positive findings by providing an overview on the data available so far on pharmacokinetics, pharmacodynamics, and analytical approaches for their detection.

References

Ameline A, Richeval C, Gaulier J-M, Raul J-S, Kintz P (2018) Characterization of flunitrazolam, a new designer benzodiazepine, in oral fluid after a controlled single administration. J Anal Toxicol. https://doi.org/10.1093/jat/bky01

Archer GA, Sternbach LH (1968) Chemistry of benzodiazepines. Chem Rev 68:747–784

Barnard EA, Darlison MG, Seeburg P (1987) Molecular biology of the GABAA receptor: the receptor/channel superfamily. Trends Neurosci 10:502–509

Baselt RC (2011) Disposition of toxic drugs and chemicals in man, 9th edn. Biomedical Publications, Seal Beach

Bauer TM, Ritz R, Haberthür C, Haefeli W, Scollo-Lavizzari G, Ha H, Hunkeler W, Sleight A (1995) Prolonged sedation due to accumulation of conjugated metabolites of midazolam. Lancet 346:145–147

Bo LD, Marcucci F, Mussini E, Perbellini D, Castellani A, Fresia P (1980) Plasma levels of chlorodesmethyldiazepam in humans. Biopharm Drug Dispos 1:123–126

Bönisch H (2007) Pharmakologie der Benzodiazepine: Allosterische Aktivatoren an GABAA-Rezeptoren. Pharm Unserer Zeit 36:186–194

Breimer DD (1979) Pharmacokinetics and metabolism of various benzodiazepines used as hypnotics. Br J Clin Pharmacol 8:7S–13S

Chebib M, Johnston GA (2000) GABA-activated ligand gated ion channels: medicinal chemistry and molecular biology. J Med Chem 43:1427–1447

Chouinard G (2004) Issues in the clinical use of benzodiazepines: potency, withdrawal, and rebound. J Clin Psychiatry 65:7–12

Coller JK, Somogyi AA, Bochner F (1999) Flunitrazepam oxidative metabolism in human liver microsomes: involvement of CYP2C19 and CYP3A4. Xenobiotica 29:973–986

El Balkhi S, Chaslot M, Picard N, Dulaurent S, Delage M, Mathieu O, Saint-Marcoux F (2017) Characterization and identification of eight designer benzodiazepine metabolites by incubation with human liver microsomes and analysis by a triple quadrupole mass spectrometer. Int J Legal Med 131:979–988

EMCDDA (2017) European drug report 2017: trends and developments. Publications Office of the European Union, Lisbon

EMCDDA (2018) Early warning system alert. Accessible via EDND database of the EMCDDA, Lisbon

Fracasso C, Confalonieri S, Garattini S, Caccia S (1991) Single and multiple dose pharmacokinetics of etizolam in healthy subjects. Eur J Clin Pharmacol 40:181–185

Franz F, Angerer V, Jechle H, Pegoro M, Ertl H, Weinfurtner G, Janele D, Schlögl C, Friedl M, Gerl S, Mielke R, Zehnle R, Wagner M, Moosmann B, Auwärter V (2017) Immunoassay screening in urine for synthetic cannabinoids – an evaluation of the diagnostic efficiency. Clin Chem Lab Med 55:1375–1384

Fraser AD, Isner AF, Bryan W (1993) Urinary screening for adinazolam and its major metabolites by the Emit® d.a.u:™ and FPIA benzodiazepine assays with confirmation by HPLC. J Anal Toxicol 17:427–431

Gavish M, Bachman I, Shoukrun R, Katz Y, Veenman L, Weisinger G, Weizman A (1999) Enigma of the peripheral benzodiazepine receptor. Pharmacol Rev 51:629–650

Golovenko NY, Larionov V (2014) Pharmacodynamical and neuroreceptor analysis of the permeability of the blood-brain barrier for derivatives of 1,4-benzodiazepine. Neurophysiology 46:199–205

Gorski JC, Jones DR, Hamman MA, Wrighton SA, Hall SD (1999) Biotransformation of alprazolam by members of the human cytochrome P4503A subfamily. Xenobiotica 29:931–944
Greenblatt D (1981) Clinical pharmacokinetics of oxazepam and lorazepam. Clin Pharmacokinet 6:89–105
Greenblatt DJ, Schillings RT, Kyriakopoulos AA, Shader RI, Sisenwine SF, Knowles JA, Ruelius HW (1976) Clinical pharmacokinetics of lorazepam. I. Absorption and disposition of oral 14C-lorazepam. Clin Pharmacol Ther 20:329–341
Hester JB, Von Voigtlander P (1979) 6-Aryl-4H-s-triazolo[4,3-a][1,4]benzodiazepines. Influence of 1-substitution on pharmacological activity. J Med Chem 22:1390–1398
Hester JB, Rudzik AD, Kamdar BV (1971) 6-Phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepines which have central nervous system depressant activity. J Med Chem 14:1078–1081
Høiseth G, Tuv SS, Karinen R (2016) Blood concentrations of new designer benzodiazepines in forensic cases. Forensic Sci Int 268:35–38
Hümpel M, Illi V, Milius W, Wendt H, Kurowski M (1979) The pharmacokinetics and biotransformation of the new benzodiazepine lormetazepam in humans I. Absorption, distribution, elimination and metabolism of lormetazepam-5-^{14}C. Eur J Drug Metab Pharmacokinet 4: 237–243
Huppertz L, Bisel P, Westphal F, Franz F, Auwärter V, Moosmann B (2015) Characterization of the four designer benzodiazepines clonazolam, deschloroetizolam, flubromazolam, and meclonazepam, and identification of their in vitro metabolites. Forensic Toxicol 33:388–395
Huppertz LM, Moosmann B, Auwärter V (2018) Flubromazolam – basic pharmacokinetic evaluation of a highly potent designer benzodiazepine. Drug Test Anal 10:206–211
Hyland R, Osborne T, Payne A, Kempshall S, Logan YR, Ezzeddine K, Jones B (2009) In vitro and in vivo glucuronidation of midazolam in humans. Br J Clin Pharmacol 67:445–454
Kilicarslan T, Haining RL, Rettie AE, Busto U, Tyndale RF, Sellers EM (2001) Flunitrazepam metabolism by cytochrome P450s 2C19 and 3A4. Drug Metab Dispos 29:460–465
Kintz P, Jamey C, Ameline A, Richeval C, Raul J-S (2017a) Characterization of metizolam, a designer benzodiazepine, in alternative biological specimens. Toxicol Anal Clin 29:57–63
Kintz P, Richeval C, Jamey C, Ameline A, Allorge D, Gaulier JM, Raul JS (2017b) Detection of the designer benzodiazepine metizolam in urine and preliminary data on its metabolism. Drug Test Anal 9:1026–1033
Kitagawa H, Esumi Y, Kurosawa S, Sekine S, Yokoshima T (1979) Metabolism of 8-chloro-6-(o-chlorophenyl)-1-methyl- 4H-s-triazolo [4,3-a] [1,4] benzodiazepine, triazolam, a new central depressant. I. Absorption, distribution and excretion in rats, dogs and monkeys. Xenobiotica 9: 415–428
Kleemann A, Engel J, Kutscher B, Reichert D (2014) Pharmaceutical substances, 5th Edition, 2009: Syntheses, Patents and Applications of the most relevant APIs. Thieme, Stuttgart
Lahti RA, Sethy VH, Barsuhn C, Hester JB (1983) Pharmacological profile of the antidepressant adinazolam, a triazolobenzodiazepine. Neuropharmacology 22:1277–1282
Lim WJ, Yap AT, Mangudi M, Koh H, Tang AS, Chan K (2017) Detection of phenazepam in illicitly manufactured Erimin 5 tablets. Drug Test Anal 9:293–305
Łukasik-Głębocka M, Sommerfeld K, Teżyk A, Zielińska-Psuja B, Panieński P, Żaba C (2016) Flubromazolam – a new life-threatening designer benzodiazepine. Clin Toxicol 54:66–68
Manchester KR, Maskell PD, Waters L (2018) Experimental versus theoretical log D7.4, pKa and plasma protein binding values for benzodiazepines appearing as new psychoactive substances. Drug Test Anal. https://doi.org/10.1002/dta.2387
Masica AL, Mayo G, Wilkinson GR (2004) In vivo comparisons of constitutive cytochrome P450 3A activity assessed by alprazolam, triazolam, and midazolam. Clin Pharmacol Ther 76: 341–349
Maskell PD, De Paoli G, Nitin Seetohul L, Pounder DJ (2012) Phenazepam: the drug that came in from the cold. J Forensic Legal Med 19:122–125
Mattila MAK, Larni HM (1980) Flunitrazepam: a review of its pharmacological properties and therapeutic use. Drugs 20:353–374

Meng L, Zhu B, Zheng K, Fu S (2017) Ultrasound-assisted low-density solvent dispersive liquid-liquid microextraction for the determination of 4 designer benzodiazepines in urine samples by gas chromatography-triple quadrupole mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 1053:9–15

Meyer MR, Bergstrand MP, Helander A, Beck O (2016) Identification of main human urinary metabolites of the designer nitrobenzodiazepines clonazolam, meclonazepam, and nifoxipam by nano-liquid chromatography-high-resolution mass spectrometry for drug testing purposes. Anal Bioanal Chem 408:3571–3591

Mohsin N, Qadir M (2015) Recent structure activity relationship studies of 1,4-benzodiazepines. Peertechz J Med Chem Res 1(1):008–0012

Moosmann B, Huppertz LM, Hutter M, Buchwald A, Ferlaino S, Auwärter V (2013a) Detection and identification of the designer benzodiazepine flubromazepam and preliminary data on its metabolism and pharmacokinetics. J Mass Spectrom 48:1150–1159

Moosmann B, Hutter M, Huppertz L, Ferlaino S, Redlingshöfer L, Auwärter V (2013b) Characterization of the designer benzodiazepine pyrazolam and its detectability in human serum and urine. Forensic Toxicol 31:263–271

Moosmann B, Bisel P, Auwärter V (2014) Characterization of the designer benzodiazepine diclazepam and preliminary data on its metabolism and pharmacokinetics. Drug Test Anal 6: 757–763

Moosmann B, King LA, Auwärter V (2015) Designer benzodiazepines: a new challenge. World Psychiatry 14:248

Moosmann B, Bisel P, Franz F, Huppertz LM, Auwärter V (2016) Characterization and in vitro phase I microsomal metabolism of designer benzodiazepines – an update comprising adinazolam, cloniprazepam, fonazepam, 3-hydroxyphenazepam, metizolam and nitrazolam. J Mass Spectrom 51:1080–1089

Mortelé O, Vervliet P, Gys C, Degreef M, Cuykx M, Maudens K, Covaci A, van Nuijs AL, Lai FY (2018) In vitro phase I and phase II metabolism of the new designer benzodiazepine cloniprazepam using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. J Pharm Biomed Anal 153:158–167

Nakamae T, Shinozuka T, Sasaki C, Ogamo A, Murakami-Hashimoto C, Irie W, Terada M, Nakamura S, Furukawa M, Kurihara K (2008) Case report: etizolam and its major metabolites in two unnatural death cases. Forensic Sci Int 182:e1–e6

Noble C, Mardal M, Bjerre Holm N, Stybe Johansen S, Linnet K (2017) In vitro studies on flubromazolam metabolism and detection of its metabolites in authentic forensic samples. Drug Test Anal 9:1182–1191

O'Connor LC, Torrance HJ, McKeown DA (2015) ELISA detection of phenazepam, etizolam, pyrazolam, flubromazepam, diclazepam and delorazepam in blood using Immunalysis® Benzodiazepine Kit. J Anal Toxicol 40:159–161

Olsen RW, Sieghart W (2008) International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric acidA receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol Rev 60:243–260

Peter R, Wicht A, Moosmann B, Huppertz L-M, Kempf J, Joly R (2017) Semi-quantitative determination of designer benzodiazepines in serum by adaption of an automated LC-MSn screening approach. Toxicol Anal Clin 29:S59–S60

Pettersson Bergstrand M, Helander A, Beck O (2016) Development and application of a multi-component LC-MS/MS method for determination of designer benzodiazepines in urine. J Chromatogr B Analyt Technol Biomed Life Sci 1035:104–110

Pettersson Bergstrand M, Helander A, Hansson T, Beck O (2017a) Detectability of designer benzodiazepines in CEDIA, EMIT II Plus, HEIA, and KIMS II immunochemical screening assays. Drug Test Anal 9:640–645

Pettersson Bergstrand M, Meyer MR, Beck O, Helander A (2017b) Human urinary metabolic patterns of the designer benzodiazepines flubromazolam and pyrazolam studied by liquid

chromatography-high resolution mass spectrometry. Drug Test Anal. https://doi.org/10.1002/dta.2243

Rudolph W, Remane D, Moosmann B, Lemke S, Auwärter V, Peters FT (2015) First reported intoxication involving the four designer benzodiazepines, flubromazepam, diclazepam, pyrazolam, and etizolam. Toxichem Krimtech 82:91

Sanna E, Pau D, Tuveri F, Massa F, Maciocco E, Acquas C, Floris C, Fontana SN, Maira G, Biggio G (1999) Molecular and neurochemical evaluation of the effects of etizolam on GABAA receptors under normal and stress conditions. Arzneimittelforschung 49:88–95

Schwartz MA, Koechlin BA, Postma E, Palmer S, Krol G (1965) Metabolism of diazepam in rat, dog, and man. J Pharmacol Exp Ther 149:423–435

Sternbach LH (1971) 1,4-Benzodiazepines. Chemistry and some aspects of the structure-activity relationship. Angew Chem Int Ed 10:34–43

Sternbach LH (1979) The benzodiazepine story. J Med Chem 22:1–7

Sternbach LH, Fryer RI, Metlesics W, Reeder E, Sach G, Saucy G, Stempel A (1962a) Quinazolines and 1,4-benzodiazepines. VI.1a halo-, methyl-, and methoxy-substituted 1,3-Dihydro-5-phenyl-2H-1,4-benzodiazepin-2-ones1b,c. J Org Chem 27:3788–3796

Sternbach LH, Fryer RI, Metlesics W, Sach G, Stempel A (1962b) Quinazolines and 1,4-benzodiazepines. V. o-aminobenzophenones1a,b. J Org Chem 27:3781–3788

Sternbach LH, Randall LO, Banziger R, Lehr H (1968) Structure-activity relationships in the 1,4-benzodiazepine series. In: Burger A (ed) Drugs affecting the central nervous system, vol 2. Edward Arnold, London

Švidrnoch M, Boráňová B, Tomková J, Ondra P, Maier V (2018) Simultaneous determination of designer benzodiazepines in human serum using non-aqueous capillary electrophoresis – tandem mass spectrometry with successive multiple ionic – polymer layer coated capillary. Talanta 176:69–76

Tomkova J, Svidrnoch M, Maier V, Ondra P (2017) Analysis of selected designer benzodiazepines by ultra high performance liquid chromatography with high-resolution time-of-flight mass spectrometry and the estimation of their partition coefficients by micellar electrokinetic chromatography. J Sep Sci 40:2037–2044

Tripsit (2018.) https://tripsit.me/. Accessed 1 Feb 2018

UNODC (1997) Recommended methods for the detection and assay of barbiturates and benzodiazepines in biological specimens. Manual for use by National Laboratories. UN document ID number: ST/NAR/28 edn. United Nations Office on Drugs and Crime, New York

UNODC (2018) Thirty-seventh meeting of the Expert Committee on Drug Dependence. http://www.who.int/medicines/access/controlled-substances/ecdd_37_meeting/en/. Accessed 1 Feb 2018

Vikingsson S, Wohlfarth A, Andersson M, Green H, Roman M, Josefsson M, Kugelberg FC, Kronstrand R (2017) Identifying metabolites of meclonazepam by high-resolution mass spectrometry using human liver microsomes, hepatocytes, a mouse model, and authentic urine samples. AAPS J 19:736–742

Waters L, Manchester KR, Maskell PD, Haider S, Haegeman C (2017) The use of a quantitative structure-activity relationship (QSAR) model to predict GABA-A receptor binding of newly emerging benzodiazepines. Sci Justice. https://doi.org/10.1016/j.scijus.2017.12.004

Wohlfarth A, Vikingsson S, Roman M, Andersson M, Kugelberg FC, Green H, Kronstrand R (2017) Looking at flubromazolam metabolism from four different angles: metabolite profiling in human liver microsomes, human hepatocytes, mice and authentic human urine samples with liquid chromatography high-resolution mass spectrometry. Forensic Sci Int 274:55–63

Zherdev VP, Caccia S, Garattini S, Ekonomov AL (1982) Species differences in phenazepam kinetics and metabolism. Eur J Drug Metab Pharmacokinet 7:191–196

Bioanalytical Methods for New Psychoactive Substances

Lea Wagmann and Hans H. Maurer

Contents

Abstract

Bioanalysis of new psychoactive substances (NPS) is very challenging due to the growing number of compounds with new chemical structures found on the drugs of abuse market. Screening, identification, and quantification in biosamples are needed in clinical and forensic toxicology settings, and these procedures are more challenging than the analysis of seized drug material because of extremely low concentrations encountered in biofluids but also due to diverse metabolic alterations of the parent compounds. This article focuses on bioanalytical single- and multi-analyte procedures applicable to a broad variety of NPS in various biomatrices, such as blood, urine, oral fluid, or hair. Sample preparation, instrumentation, detection modes, and data evaluation are discussed as well as corresponding pitfalls. PubMed-listed and English-written original research papers and review articles published online between 01 October 2012 and 30 September 2017 were considered.

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Bioanalysis · Biosamples · Blood · Detection · Drugs of abuse · Hair · Mass spectrometry · New psychoactive substances · Novel psychoactive substances · NPS · Oral fluid · Plasma · Quantification · Screening · Serum · Urine

Acronyms of the Discussed New Psychoactive Substances (NPS)

1P-LSD	1-Propionyl-lysergic acid diethylamide
25B-NBOMe	2-(4-Bromo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl) methyl]ethanamine
25C-NBOMe	2-(4-Chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl) methyl]ethanamine
25E-NBOMe	2-(4-Ethyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl) methyl]ethanamine
25H-NBOMe	2-(2,5-Dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine
25I-NBOMe	2-(4-Iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl) methyl]ethanamine
2C	2,5-Dimethoxyphenylethylamine
3-FPM	3-Fluorophenmetrazine
3-MeO-PCP	3-Methoxyphencyclidine
3-MMC	3-Methylmethcathinone
4-MeO-PCP	4-Methoxyphencyclidine
5F-APINACA	*N*-(1-Adamantyl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide
5F-MDMB-PICA	Methyl-*N*-{[1-(5-fluoropentyl)-1*H*–indol-3-yl]carbonyl}-3-methylvalinate
5-IT	5-(2-Aminopropyl)indole
α-PVP	α-Pyrrolidinopentiophenone
α-PVT	α-Pyrrolidinopentiothiophenone
AB-FUBINACA	*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide
AL-LAD	N^6-Allyl-6-norlysergic acid diethylamide
AMB-FUBINACA	Methyl-*N*-[1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamido]-3-methylbutanoate
APINACA	*N*-(1-Adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide
LSZ	(2′*S*,4′*S*)-Lysergic acid 2,4-dimethylazetidide
MDMB-CHMICA	Methyl-*N*-{[1-(cyclohexylmethyl)-1*H*-indol-3-yl]carbonyl}-3-methylvalinate
MDPV	3,4-Methylenedioxypyrovalerone
MEC	Methylethcathinone
MMC	Methylmethcathinone
MT-45	1-Cyclohexyl-4-(1,2-diphenylethyl)piperazine
PV8	α-Pyrrolidinoenanthophenone

U-47700	3,4-Dichloro-*N*-[2-(dimethylamino)cyclohexyl]-*N*-methylbenzamide
UR-144	1-Pentyl-1*H*-indol-3-yl-(2,2,3,3-tetramethylcyclo-propyl) methanone

1 Introduction

Bioanalysis of new psychoactive substances (NPS) is very challenging due to the growing number of compounds available on the drugs of abuse market. In Europe, the number of NPS notified by the European Union (EU) Early Warning System coordinated by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has risen rapidly since 2008 (EMCDDA 2017). More than 700 NPS from different chemical and pharmacological classes are continuously monitored as part of the EMCDDA's early-warning and toxicovigilance system. A significant proportion of detected substances are synthetic cathinones or synthetic cannabinoid receptor agonists (SCRAs), but synthetic opioids and benzodiazepines are on the rise since 2014. The first indication of a newly emerging compound on the market often arises from its identification in seized samples or from test purchases. Recommendations on such kind of analysis are published, for example, by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG 2016). NPS initially circumvent existing legislation and are usually offered as legal alternative to traditional and thus controlled drugs of abuse. Consumers can easily obtain them, e.g., via Internet retailers, labeled, for instance, as research chemicals, plant food, or bath salts, often with the addition "not for human consumption." The Internet provides a rich source of information for consumers as well as for healthcare professionals or policy makers (Deluca et al. 2012). Even though NPS are initially not controlled and easily available, information about potentially harmful effects is frequently available (Logan et al. 2017; Nelson et al. 2014; Zamengo et al. 2014).

In the following, an overview is provided of recent developments in the field of bioanalytical methods of analysis applied to a variety of NPS including information on sample preparation, instrumentation, detection modes, data evaluation, and pitfalls. PubMed-listed and English-written original research papers and review articles published during 01 October 2012 and 30 September 2017 were considered including single- and multi-analyte procedures.

2 Methods

Literature research was performed in PubMed (National Center for Biotechnology Information, US National Library of Medicine, Bethesda, MD, USA) for English-written articles published online between 01 October 2012 and 30 September 2017. The search terms "new psychoactive substances" or "novel psychoactive

substances" in combination with one of the key words "screening," "detection," "quantification," "bioanal*," "mass spectrom*," or "quantification" in any field were used: ((new psychoactive substances OR novel psychoactive substances) AND (screening OR detection OR quantification OR bioanal* OR mass spectrom*)) AND ("2012/10/01" [PDAT]: "2017/09/30" [PDAT]). Both review articles and original research papers were considered but single case reports only if they were the only information source concerning the intake of a specific NPS and if an analytical confirmation was described.

3 General Aspects on Bioanalysis of NPS

Screening, identification, and quantification of NPS in different biosamples such as blood, urine, hair, oral fluid, and tissue samples are important in the clinical and forensic toxicology environment (Peters 2014). Biosampling strategies used for matrix alternatives to classic blood and urine were reviewed elsewhere (Mercolini and Protti 2016). Urine is the preferred matrix in various areas of analytical toxicology, such as abstinence monitoring, workplace drug testing, or where comprehensive screening purposes are required, as it can be obtained noninvasively in comparable large volumes and all substances and/or their metabolites are concentrated (Meyer and Maurer 2016; Peters 2014). Especially in point-of-care drug testing, immunoassay screening commonly provides a first indication of the presence of drugs (of abuse). However, only few immunoassays applicable to the detection of NPS were developed (Arntson et al. 2013; Barnes et al. 2015; Castaneto et al. 2015a; Mohr et al. 2014). A considerable challenge stems from the multiplicity of substances and chemical structures available but also from the time-consuming production of antibodies that takes often more time than the "half-life" of a specific NPS on the market (Favretto et al. 2013). More promising could be a structure-independent assay system recently reported for activity profiling of synthetic cannabinoids developed by Cannaert et al. (2016). They developed cell systems stably expressing the cannabinoid receptors CB1 or CB2 connected to a luciferase reporter system. The assay allowed for the measurement of receptor activation via β-arrestin involvement and bioluminescence, which was successfully applied to authentic urine samples obtained from users of SCRAs (Cannaert et al. 2017). This approach is independent of the compound structure but requires excretion of active substances into urine. Furthermore, phytocannabinoids are also detected, and further identification and confirmation steps are needed, preferably by mass spectrometry (MS). In immunoassays designed for detection of traditional drugs, some NPS might give positive results due to cross-reactivity, but that is dependent on the NPS class and concentration, which means that they are not identified reliably (Beck et al. 2014; Nieddu et al. 2016; O'Connor et al. 2016; Pettersson Bergstrand et al. 2017a; Swortwood et al. 2014).

Recent literature is consistent in the recommendation to use MS-based methods for NPS screening (Al-Saffar et al. 2013; Favretto et al. 2013; Franz et al. 2017a; Sundstrom et al. 2015). Only MS, usually deployed after implementation of a variety

of separation methods, provides the high level of flexibility, sensitivity, and selectivity that is needed for robust and reliable detection of NPS (Meyer and Maurer 2016). However, lipophilic NPS are extensively metabolized, and thus, the analytical strategy has to consider metabolites as targets particularly in urine. According to several national guidelines, e.g., in Sweden, metabolite detection needs confirmation using reference substances, and the use of mass spectral reference libraries also containing the metabolites is highly recommended (Helfer et al. 2015; Wissenbach et al. 2011). In contrast to licensed therapeutic drugs, pharmacokinetic data for NPS are not available. Thus, metabolism studies are mandatory for developing screening methods. Metabolite data can be generated by using in vitro or in vivo models, or metabolites can be identified in samples obtained from authentic cases (Maurer and Meyer 2016; Richter et al. 2017; Schaefer et al. 2016; Welter-Luedeke and Maurer 2016). Further information can be found in the review and the corresponding chapter of this handbook by Meyer summarizing toxicodynamics and toxicokinetics of NPS (Meyer 2016).

MS-based screening procedure can be divided into targeted and untargeted procedures (Meyer and Maurer 2016). Targeted screenings, often combined with quantification, focus on a predefined set of analytes and are traditionally performed with low-resolution tandem mass spectrometry (MS/MS) devices using selected reaction monitoring (SRM) mode providing high selectivity and sensitivity. Covering only the targets, such procedures cannot detect unexpected or unknown compounds. In contrast, untargeted full scan screenings cover all analytes contained in the used library and allow retrospective data mining. They can also give indications of new compounds if they can be extracted, separated, and ionized. High-resolution (HR) MS/MS data often help to get an idea about the structure, particularly if known partial structures are indicated. Of course, the identity must be confirmed by reference standards. HRMS can also be used for data-independent acquisition (DIA), where all product ions are recorded regardless of the precursor ion, and data-dependent acquisition (DDA) mode, where preset criteria define the precursor ions for MS/MS spectral recording. Sundstrom et al. compared post-targeted DIA and pre-targeted DDA for urine drug screening based on quadrupole time-of-flight (QTOF) MS and discovered that DIA was more straightforward and the method was easier to deploy in casework and that the DDA approach with substance-specific collision energies produced informative product ion spectra suitable for occasional confirmatory analyses (Sundstrom et al. 2017).

4 Bioanalysis of NPS of Different Classes

Some methods for simultaneous detection of a broad range of NPS of different classes were published, and details are given in Table 1. Mainly stimulants but also SCRAs and hallucinogens were included in such targeted multi-analyte approaches. The blood screening procedure described by Adamovicz and Tokarczyk covered 143 NPS with a rapid and simple sample preparation and limits of detection (LOD) estimated for 104 compounds in the range 0.01–3.09 ng/mL (Adamowicz and

Table 1 Biosamples, experimental setups, and highlights of multi-analyte approaches for detection of NPS of different classes

Number of NPS	Biosample	Workup	Instrumentation	Detection mode	Highlights	Reference
143 NPS (incl. 58 stimulants, 34 SCRAs, and 25 hallucinogens)	B	PRE	LC-MS/MS	SRM	Rapid and simple sample preparation	Adamowicz and Tokarczyk (2016)
64 NPS	B	PRE	LC-MS/MS	SRM	Rapid and simple sample preparation, quantitative procedure	Vaiano et al. (2016)
69 NPS	S	SPE	LC-MS/MS	SRM	Fully validated for 69 NPS, quantitative procedure	Lehmann et al. (2017)
25 NPS	P	PRE	LC-OT-MS/MS	Full scan and t-SIM	Screening and confirmation in one analytical run, quantitative procedure	Montesano et al. (2016)
78 NPS	B	DLLME	LC-MS/MS	SRM	DLLME as suitable alternative sample preparation for blood	Odoardi et al. (2015)
64 NPS (incl. >30 stimulants and 7 tryptamines)	DBS	ME	LC-MS/MS	SRM	DBS shown to be a suitable alternative sampling strategy in NPS analytics	Ambach et al. (2014)
11 NPS (56 analytes in total)	U	LLE	LC-MS/MS	SRM	Simultaneous identification and quantification of traditional drugs of abuse, benzodiazepines, and NPS	Lee et al. (2016)
44 NPS, mainly stimulants	U	GRD-SPE	LC-MS/MS	SRM	Simultaneous detection of conventional and new drugs of abuse	Tang et al. (2014)
26 NPS, mainly stimulants	U	DIL	LC-MS/MS	SRM	Rapid and simple sample preparation, quantitative procedure	Al-Saffar et al. (2013)
120 NPS	U	DIL	LC-OT-MS/MS	Full scan-XIC, PRM for confirmation	Discussed as “black-box” solution and replacement of immunochemical urine drug testing, quantitative procedure	Stephanson et al. (2017)
132 NPS	H	Acidic ME	LC-MS/MS	SRM	One-step extraction from pulverized hair	Boumba et al. (2017)

50 NPS	H	ME or acidic WE	LC-MS/MS	SRM	Sample preparation dependent on target NPS class, quantitative procedure	Strano-Rossi et al. (2014)
14 NPS	H	PLE-SPE	LC-OT-MS/MS	Full scan and t-SIM	Retrospective data mining possible, quantitative procedure	Montesano et al. (2017)

B whole blood, *DBS* dried blood spots, *DIL* dilution, *DLLME* dispersive liquid-liquid microextraction, *GRD* β-glucuronidase, *H* hair, *LC* liquid chromatography, *LLE* liquid-liquid extraction, *ME* extraction with methanol, *MS/MS* tandem mass spectrometry, *OT* orbitrap, *P* plasma, *PLE* pressurized liquid extraction, *PRE* precipitation, *PRM* product reaction monitoring, *S* serum, *SCRAs* synthetic cannabinoid receptor agonists, *SPE* solid-phase extraction, *SRM* selected reaction monitoring, *t-SIM* targeted selected ion monitoring, *U* urine, *WE* extraction with water, *XIC* extracted ion chromatograms

Tokarczyk 2016). Vaiano et al. included 64 NPS in their method for blood prepared by protein precipitation (Vaiano et al. 2016). Lehmann et al. used automated solid-phase extraction (SPE) for detection of 69 NPS in serum, and total cycle time for one sample was only 11 min due to the interlacing between sample preparation and analysis (Lehmann et al. 2017). Odoardi et al. applied dispersive liquid-liquid microextraction using minimal amount of organic solvent as rapid, cheap, and efficient alternative sample preparation for blood (Odoardi et al. 2015). Ambach et al. successfully used dried blood spots as an alternative sampling strategy to screen for 64 NPS and also reported about substance stability in dried blood spots (Ambach et al. 2014). Three studies focusing on targeted screening in urine were identified, but unfortunately, only parent compounds and no metabolites were included, which is insufficient for comprehensive urine screening procedures as discussed earlier (Al-Saffar et al. 2013; Lee et al. 2016; Tang et al. 2014). Boumba et al. described a procedure for analysis of 132 NPS in hair, which provided LOD from 0.001 to 0.1 ng/mg hair (Boumba et al. 2017). LOD were comparable to them described in other studies (Montesano et al. 2017; Strano-Rossi et al. 2014).

The majority of these multi-analyte approaches were targeted screenings based on liquid chromatography (LC) coupled to MS/MS operating in SRM mode with triple quadrupole or quadrupole ion trap hybrid mass analyzers. Only three screening procedures based on HRMS were described (Montesano et al. 2016, 2017; Stephanson et al. 2017). Stephanson et al. performed first compound detection by extracted ion chromatograms from full scan and second confirmation by product reaction monitoring. Only in case of interferences in full scan, product reaction monitoring was already used as first step for some NPS (Stephanson et al. 2017). The studies published by Montesano et al. used both, targeted selected-ion monitoring and full scan (Montesano et al. 2016, 2017). The application of full scan also allowed for retrospective data mining.

5 Bioanalysis of NPS Stimulants

NPS acting as psychostimulants are derivatives of phenethylamine, amphetamine, or cathinone, and they belong to the most abundant representatives on the NPS market in the EU (Tyrkko et al. 2016). Some reviews summarized the existing knowledge about stimulants including analytical methods and detectability. Whereas Welter-Luedeke and Maurer focused on amphetamine derivatives with modified ring systems, Ellefsen et al. were interested in synthetic cathinones (Ellefsen et al. 2016; Welter-Luedeke and Maurer 2016). Alvarez et al. published a review about hair analysis of synthetic cathinones (Alvarez et al. 2017). Details of original research papers focusing on bioanalysis of stimulants can be found in Table 2. As part of the Swedish STRIDA project, five studies summarizing serum and urine concentrations of 15 different stimulants were published (Backberg et al. 2015c, 2016; Beck et al. 2015, 2017). The STRIDA project was initiated to monitor the occurrence and health hazards associated with NPS consumption in Sweden. Information about clinical effects originated from medical record data in laboratory

Table 2 Biosamples, experimental setups, and highlights of studies containing analytical methods for detection of stimulants

NPS	Biosample	Workup	Instrumentation	Detection mode	Highlights	Reference
3-MMC	S, U	PRE, DIL	LC-MS/MS	SRM	3-MMC serum and urine concentrations of 50 cases	Backberg et al. (2015c)
MDPV	S, U	PRE, DIL	LC-MS/MS	SRM	MDPV serum and urine concentrations of 201 cases	Beck et al. (2015)
α-PVP	S, U	PRE, DIL	LC-MS/MS, LC-OT-MS/MS	SRM, full scan	α-PVP serum and urine concentrations of 42 cases	Beck et al. (2016)
3-FPM	S, U	PRE, DIL	LC-MS/MS, LC-HRMS/MS	SRM, full scan	3-FPM serum and urine concentrations of 19 cases	Backberg et al. (2016)
11 MDPV derivatives	S, U	PRE, DIL	LC-MS/MS, LC-OT-MS/MS	SRM, full scan	NPS serum and urine concentrations of 114 cases	Beck et al. (2017)
3-MMC	B	LLE	LC-MS/MS	SRM	3-MMC blood concentrations of 95 cases	Adamowicz et al. (2016)
MMC and MEC ortho-, meta-, para-isomers	S	PRE	LC-MS/MS	SRM	Separation of MMC and MEC positional isomers	Maas et al. (2017)
MDPV	S	SPE	GC-MS	SIM	MDPV serum concentrations of 23 cases	Grapp et al. (2017)
Mephedrone	P, U	(PRE) LLE, MSTFAD	GC-MS	SIM	Pharmacokinetic data from a pilot clinical trial with six volunteers	Olesti et al. (2017)
α-PVT	U	SPE	LC-OT-MS/MS	Full scan and DDA or AIF	Metabolism data, targets recommended for urine drug testing	Swortwood et al. (2016a)
PV8	U	SPE	LC-OT-MS/MS	Full scan and DDA	Metabolism data, targets recommended for urine drug testing	Swortwood et al. (2016b)
40 stimulants	U	SPE	LC-OT-MS/MS	Full scan and DDA	Four metabolites included, application to authentic samples	Concheiro et al. (2015)
26 stimulants	H	ME	LC-MS/MS	SRM	Simple sample preparation	Salomone et al. (2016)

(continued)

Table 2 (continued)

NPS	Biosample	Workup	Instrumentation	Detection mode	Highlights	Reference
8 stimulants	H	Acidic ME and SPE	LC-MS/MS	SRM	Two-step sample preparation	Lendoiro et al. (2017)
11 stimulants	OF	MEPS	LC-MS/MS	SRM	MEPS suitable alternative to SPE	Ares et al. (2017)
32 stimulants	OF	PRE	LC-MS/MS	SRM	Applicable for workplace drug testing of stimulant NPS	Williams et al. (2017)

AIF all-ions fragmentation, *B* whole blood, *DDA* data-dependent acquisition, *DIL* dilution, *GC* gas chromatography, *H* hair, *HRMS/MS* high-resolution tandem mass spectrometry, *LC* liquid chromatography, *LLE* liquid-liquid extraction, *ME* extraction with methanol, *MEPS* microextraction by packed sorbent, *MS/MS* tandem mass spectrometry, *MSTFAD* derivatization with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide, *OF* oral fluid, *OT* orbitrap, *P* plasma, *PRE* precipitation, *S* serum, *SIM* selected ion monitoring, *SPE* solid-phase extraction, *SRM* selected reaction monitoring, *U* urine

confirmed cases of acute drug intoxications presenting at emergency departments or intensive care units all over Sweden and were collected to extend the knowledge about NPS, drug trends, and health risks over a longer time period (Helander et al. 2014b). Adamowicz et al. interpreted 3-MMC blood concentrations in forensic context based on analysis of 95 cases (Adamowicz et al. 2016). Maas et al. considered the identification of positional isomers to be an important issue in forensic toxicology due to differences in legal status or toxicity and developed a method for the separation of *ortho*-, *meta*-, and *para*-isomers of methylmethcathinone and methylethcathinone in serum samples (Maas et al. 2017). Grapp et al. reported MDPV serum concentrations determined in 23 cases (Grapp et al. 2017). Olesti et al. developed a quantification method for mephedrone in plasma and urine and applied it to samples taken from six volunteers following oral intake of 150 mg mephedrone as part of a randomized, double-blind, crossover controlled clinical trial (Olesti et al. 2017). The metabolic fate of two α-PVP derivatives was elucidated using human hepatocyte incubations and authentic urine specimens (Swortwood et al. 2016a, b). Concheiro et al. developed a procedure for simultaneous determination of 40 stimulants in urine (Concheiro et al. 2015). Unchanged parent compounds were chosen as targets, and only four metabolites were included, which might prove insufficient for urine screening, despite implementation of SPE for concentration during sample preparation. Two studies focused on detection of stimulants in hair samples (Lendoiro et al. 2017; Salomone et al. 2016). Ares et al. presented a screening procedure for oral fluid based on microextraction by packed sorbent (MEPS) followed by LC-MS/MS. MEPS, a miniaturized version of SPE, provided some advantages including short extraction times, reduced sample and solvent consumption, and facile operation and was shown to be a suitable sample preparation method for oral fluid (Ares et al. 2017). Williams et al. also developed and validated a method for detection of synthetic cathinones in oral fluid and prepared samples by simple precipitation (Williams et al. 2017). Furthermore, various stimulants were included in multi-analyte approaches mentioned above (Adamowicz and Tokarczyk 2016; Al-Saffar et al. 2013; Ambach et al. 2014; Boumba et al. 2017; Lee et al. 2016; Lehmann et al. 2017; Montesano et al. 2016, 2017; Odoardi et al. 2015; Stephanson et al. 2017; Strano-Rossi et al. 2014; Tang et al. 2014; Vaiano et al. 2016).

6 Bioanalysis of Synthetic Cannabinoid Receptor Agonists

Together with stimulants, SCRAs form the largest group of compounds detected on the NPS market. Smokable, herbal products containing SCRAs, commonly referred to as "Spice," are believed to share some biological effects of naturally occurring phytocannabinoids (Karila et al. 2016). However, numerous adverse events and fatalities linked to their use were reported most likely caused by distinct pharmacological properties enhancing their toxic profile. On the one hand, SCRAs were described as full cannabinoid receptor CB1 and CB2 agonists, in contrast to partial

agonist properties of the primary psychoactive compound of marijuana Δ^9-tetrahydrocannabinol (THC), and on the other hand, several metabolites have been reported to retain, at least in some cases, affinity and activity at cannabinoid receptors (Fantegrossi et al. 2014). Some confusion can be caused by nomenclature of SCRAs. For example, AMB-FUBINACA, MMB-FUBINACA, and FUB-AMB are different names of the same compound (Adams et al. 2017). Structurally different from THC, neither parent compound nor SCRA metabolites are usually detected with standard cannabinoid immunoassays. Even if development and validation of specialized immunoassays are possible, this is extremely time-consuming, and due to the structural differences within the group of SCRAs, only few compounds are detected, which are already replaced by others once the immunoassay is marketable (Castaneto et al. 2015b). However, immunoassays and the already discussed bioassay for activity profiling developed by Cannaert et al. must be complemented by MS-based confirmation analyses (Cannaert et al. 2016; Castaneto et al. 2015b). Castaneto et al. published a review about SCRA pharmacokinetics and detection methods in biological matrices. They mentioned that interpretation of MS-based results should include discussion of common metabolites formed by different SCRAs. Details of original research articles focusing on bioanalysis of SCRAs can be found in Table 3. Tynon et al. developed a screening and confirmation procedure for 34 SCRAs in whole blood (Tynon et al. 2017). Karinen et al. reported SCRA concentrations measured in blood samples of drivers suspected of impaired driving in Norway (Karinen et al. 2015). Protti et al. successfully used dried hematic microsamples for detection of SCRAs (Protti et al. 2017). Adams et al. identified AMB-FUBINACA, contained in the herbal incense "AK-47 24 Karat Gold," as trigger of a mass intoxication in New York City by analyzing the incense product and biosamples of eight intoxicated individuals. However, the parent compound being a methyl ester could only be found in the incense product itself while only the de-esterified acid metabolite was detected in biosamples (Adams et al. 2017). Backberg et al. published a series of nine analytically confirmed intoxication with MDMB-CHMICA from the Swedish STRIDA project (Backberg et al. 2017). Franz et al. detected metabolites of the same SCRA in 818 authentic urine samples and analyzed furthermore in vitro incubations with human liver microsomes and smoke condensates (Franz et al. 2017b). For most SCRAs, the parent compounds are rarely detected in urine. Vikingsson et al. used authentic urine samples and incubations with human liver microsomes to identify urinary AB-FUBINACA metabolites suitable as targets for drug testing (Vikingsson et al. 2016). Mogler et al. described similar experiments for detecting 5F-MDMB-PICA metabolites (Mogler et al. 2017). Borg et al. developed an analytical method to detect metabolites of 32 SCRAs in urine specimens (Borg et al. 2017). This was the only multi-analyte approach used for urine screening that exclusively focused on metabolite detection. Salomone et al. published a screening procedure for detection of 23 SCRAs in hair covering only the parent compounds (Salomone et al. 2014). However, detecting only parent compounds complicates the differentiation of consumption from passive contamination (Castaneto et al. 2015b). Furthermore, some SCRAs or their metabolites were included in the multi-analyte approaches for NPS of different

Table 3 Biosamples, experimental setups, and highlights of studies containing analytical methods for detection of synthetic cannabinoid receptor agonists

NPS	Biosample	Workup	Instrumentation	Detection mode	Highlights	Reference
34 SCRAs	B	LLE	LC-MS/MS	SRM	Different, optimized conditions for aminocarbonyl/carboxamide or arylindole SCRAs	Tynon et al. (2017)
APINACA, 5F-APINACA, UR-144	B	LLE	LC-MS/MS	SRM	UR-144 degradant product also quantified	Karinen et al. (2015)
10 SCRAs	DBS, VAMS	ME	LC-MS/MS	SRM	VAMS tested as alternative to DBS, natural cannabinoids also included	Protti et al. (2017)
AMB-FUBINACA	S, B, U	PRE GRD-DIL	LC-QTOF-MS/MS	Auto-MS/MS	SCRA caused "zombielike" behavior and mass intoxication in New York City	Adams et al. (2017)
MDMB-CHMICA	S, U	PRE, DIL	LC-MS/MS, LC-HRMS/MS	SRM, full scan	NPS concentrations, patients' laboratory and clinical data	Backberg et al. (2017)
MDMB-CHMICA	U	GRD-SALLE	LC-MS/MS, LC-QTOF-MS/MS	EPI, PIS, SRM, full scan and auto-MS/MS	Identification of thermal degradation products identical to metabolites, important for hair analysis	Franz et al. (2017b)
AB-FUBINACA	U	GRD-SALLE	LC-QTOF-MS/MS	DDA	Identification of urinary markers for AB-FUBINACA intake	Vikingsson et al. (2016)
5F-MDMB-PICA	U	GRD-SALLE, PRE	LC-MS/MS, LC-QTOF-MS/MS	EPI, PIS, SRM, full scan and auto-MS/MS	Extensive glucuronidation of main phase I metabolites, sensitivity enhanced by conjugate cleavage	Mogler et al. (2017)
32 SCRAs	U	GRD-LLE	LC-MS/MS	SRM	Only metabolites as targets	Borg et al. (2017)
23 SCRAs	H	Incubation with NaOH, followed by LLE	LC-MS/MS	SRM	15 positives in authentic samples	Salomone et al. (2014)

B whole blood, *DBS* dried blood spots, *DDA* data-dependent acquisition, *DIL* dilution, *EPI* enhanced product ion scan, *GRD* β-glucuronidase, *H* hair, *HRMS/MS* high-resolution tandem mass spectrometry, *LC* liquid chromatography, *LLE* liquid-liquid extraction, *ME* extraction with methanol, *MS/MS* tandem mass spectrometry, *PIS* precursor ion scan, *PRE* precipitation, *QTOF* quadrupole time-of-flight, *S* serum, *SALLE* salting-out assisted liquid-liquid extraction, *SCRAs* synthetic cannabinoid receptor agonists, *SRM* selected reaction monitoring, *U* urine, *VAMS* volumetric absorptive microsampling

classes as described before (Adamowicz and Tokarczyk 2016; Boumba et al. 2017; Lee et al. 2016; Montesano et al. 2016, 2017; Odoardi et al. 2015; Strano-Rossi et al. 2014; Tang et al. 2014; Vaiano et al. 2016).

7 Bioanalysis of Synthetic NPS Opioids

In both Europe and North America, the recent emergence of new synthetic opioids, mostly fentanyl derivatives, is causing considerable concern. Due to their high potency, they present serious health risks, not only to those who use them but also to those involved in their handling. Consequently, reports about nonfatal intoxications but also deaths caused by synthetic opioids are increasingly reported since 2012 (EMCDDA 2017). Due to structural differences to morphine, a detection of synthetic opioids with standard immunoassays designed for opiates is not possible (Helander et al. 2014a). Details of studies focusing on bioanalysis of synthetic opioids can be found in Table 4. Within the framework of the STRIDA project, three studies were published that described a total of 29 intoxication cases involving 10 different fentanyl derivatives (Backberg et al. 2015b; Helander et al. 2016, 2017). The same authors also published a report about a case series of nonfatal intoxications with MT-45 (Helander et al. 2014a). Papsun et al. developed and validated a method for MT-45 quantification in whole blood and Fleming et al. for detection and quantification of U-47700 including four authentic urine samples (Fleming et al. 2017; Papsun et al. 2016). Metabolites of fentanyl derivatives as targets for urine drug testing were identified by Steuer et al. (2017) and Watanabe et al. (2017). Noble et al. developed a targeted screening method to detect 50 fentanyl analogs in whole blood using LC-QTOF-MS and offered a validation for 13 compounds (Noble et al. 2017a).

8 Bioanalysis of Designer Benzodiazepines

Over the last few years, an increasing number of benzodiazepine NPS appeared. These so-called designer benzodiazepines are structurally related to clinically used benzodiazepines (Manchester et al. 2017). Owing to this structure similarity, cross-reactivity with immunoassay antibodies was observed, and some designer benzodiazepines could be detected in standard immunoassay drug screenings of both blood and urine samples (O'Connor et al. 2016; Pettersson Bergstrand et al. 2017a). However, false negative immunoassay screening results have also been encountered (Huppertz et al. 2017; Moosmann et al. 2013, 2014). Negative immunoassay results underline the need for MS-based analysis, but analytical results have to be interpreted with care. Licensed benzodiazepines can be identical to designer benzodiazepine metabolites (e.g., clonazepam detection after intake of cloniprazepam), or the designer benzodiazepine itself can have the same structure as metabolites of prescribed benzodiazepines (e.g., fonazepam, identical to norflunitrazepam) (Moosmann et al. 2016). Isomers such as diclazepam and

Table 4 Biosamples, experimental setups, and highlights of studies containing analytical methods for detection of synthetic opioids

NPS	Biosample	Workup	Instrumentation	Detection mode	Highlights	Reference
Butyrfentanyl, 4-fluorobutyrfentanyl	S, U	PRE, DIL	LC-MS/MS, LC-HRMS/MS	SRM, full scan	NPS concentrations, case histories	Backberg et al. (2015b)
Acetylfentanyl, 4-methoxybutyrfentanyl, furanylfentanyl	S, U	PRE, DIL	LC-MS/MS, LC-HRMS/MS	SRM, full scan	NPS concentrations, patients' laboratory and clinical data	Helander et al. (2016)
Acrylfentanyl, 4-chlorisobutyrfentanyl, 4-fluoroisobutyrfentanyl, tetrahydrofuranfentanyl, cyclopentylfentanyl	S, U	PRE, DIL	LC-MS/MS, LC-HRMS/MS	SRM, full scan	NPS concentrations, patients' laboratory and clinical data	Helander et al. (2017)
MT-45	S, U	PRE, DIL	LC-MS/MS	SRM	NPS concentrations, patients' laboratory and clinical data	Helander et al. (2014a)
MT-45	B	LLE	LC-MS/MS	SRM	Validated quantification method	Papsun et al. (2016)
U-47700	U	DIL, SPE	LC-QTOF-MS/MS, LC-MS/MS	IDA, SRM	Metabolite investigation in silico and in authentic samples	Fleming et al. (2017)
Butyrfentanyl	B, U	PRE, (ARS/GRD)-SPE	LC-QTOF-MS/MS	IDA	In vitro incubations with human liver microsomes and cytochrome P450 isoforms	Steuer et al. (2017)
Acetylfentanyl, acrylfentanyl furanylfentanyl, 4-fluoroisobutyrfentanyl	U	(ARS/GRD)-DIL	LC-QTOF-MS/MS	Auto-MS/MS	In vitro incubations with human hepatocytes, targets for urine screening	Watanabe et al. (2017)
50 fentanyl analogs	U	PRE	LC-QTOF-MS/MS	DIA	Tentative identification of fentanyl analogs without reference standard	Noble et al. (2017a)

ARS arylsulfatase, *B* whole blood, *DIA* data-independent acquisition, *DIL* dilution, *GRD* β-glucuronidase, *HRMS/MS* high-resolution tandem mass spectrometry, *IDA* information-dependent acquisition, *LC* liquid chromatography, *LLE* liquid-liquid extraction, *MS/MS* tandem mass spectrometry, *PRE* precipitation, *QTOF* quadrupole time-of-flight, *S* serum, *SPE* solid-phase extraction, *SRM* selected reaction monitoring, *U* urine

4-chlorodiazepam can also lead to misidentification. Furthermore, designer benzodiazepines are possibly used as therapeutics in some countries, such as phenazepam, which was a prescription drug in the former Soviet states, and etizolam, which was originated in Japan (Manchester et al. 2017).

The review article by Manchester et al. about the emergence of designer benzodiazepines contained also an overview of analytical methods employed for their identification in biological matrices (Manchester et al. 2017). Experimental setups and highlights of original research articles containing analytical methods for detection of designer benzodiazepines are summarized in Table 5. A combined targeted and nontargeted drug screening in whole blood by HRMS with data-independent acquisition was described by Mollerup et al. (2017). First, a targeted screening was performed with identification based on reference standard data. However, the authors stated that due to the continuous appearance of NPS, it is almost impossible to generate all reference standard data and to keep the analytical method updated permanently. To overcome this shortcoming, a subsequent non-targeted screening extracted information regarding previously unidentified peaks for additional drug identifications and metabolite confirmation. To test its applicability, blood was spiked with 11 low-dosed designer benzodiazepines, and 9 were tentatively identified in the nontargeted screening approach in the concentration range 0.005–0.1 mg/kg. Hoiseth et al. published blood concentrations of five designer benzodiazepines detected in 77 authentic samples of drugged drivers or other criminal offenders (Hoiseth et al. 2016). After a first screening for a broad repertoire of drugs of abuse and medicines, a second confirmation analysis with quantification was performed. The authors stated that reported concentrations could be helpful in future interpretation and assessment of designer benzodiazepine blood concentrations in forensic toxicology.

As previously discussed, metabolites have to be considered in urine screening (Manchester et al. 2017). Several studies describing detection of designer benzodiazepines in urine provided also information about formed metabolites (Huppertz et al. 2017; Meyer et al. 2016; Moosmann et al. 2013, 2014; Noble et al. 2017b; Pettersson Bergstrand et al. 2017b; Vikingsson et al. 2017). The studies performed by Moosmann et al. in 2013 and 2014 and Huppertz et al. in 2017 were very similar in their experimental setup but differed in the investigated compound. Each of them contained a self-experiment by one of the authors, and serum concentrations and metabolites in urine were described as well as elimination half-life and results of immunoassay screening (Huppertz et al. 2017; Moosmann et al. 2013, 2014). Noble et al. investigated the metabolic fate of flubromazolam by means of in vitro incubations with pooled human liver microsomes or recombinant cytochrome P450 isoforms. Metabolites were confirmed by analyses of authentic samples obtained from two forensic cases (Noble et al. 2017b). Vikingsson et al. used mice urine and human hepatocytes in addition to microsomes and authentic human urine samples to identify meclonazepam metabolites. Similar to therapeutic nitro-containing benzodiazepines, amino-meclonazepam and acetamino-meclonazepam were found to be the main metabolites in human urine, which were also found in mice urine and human primary hepatocytes. However, human liver microsomes

Table 5 Biosamples, experimental setups, and highlights of studies containing analytical methods for detection of designer benzodiazepines

NPS	Biosample	Workup	Instrumentation	Detection mode	Highlights	Reference
11 designer benzodiazepines	B (spiked)	PRE	LC-QTOF-MS	DIA	Non-targeted screening	Mollerup et al. (2017)
Clonazolam, diclazepam, flubromazepam, flubromazolam, pyrazolam	B	LLE	LC-MS/MS	SRM	Blood concentrations of 77 authentic cases	Hoiseth et al. (2016)
Flubromazepam	S, U	LLE, GRD-LLE	LC-MS/MS	SRM	Pharmacokinetic data, serum concentrations after oral intake of 4 mg	Moosmann et al. (2013)
Diclazepam	S, U	LLE, GRD-LLE	LC-MS/MS	SRM	Pharmacokinetic data, serum concentrations after oral intake of 1 mg	Moosmann et al. (2014)
Flubromazolam	S, U, H	(ARS/GRD)-LLE, ME	LC-MS/MS or -MS^3 (for H)	SRM	Pharmacokinetic data, serum concentrations after oral intake of 0.5 mg	Huppertz et al. (2017)
Flubromazolam	S, T, U	PRE, GRD-PRE	LC-OT-MS/MS and LC-MS/MS	Full scan and $ddMS^2$ or PRM and SRM	Metabolism studies, targets recommended for urine drug testing	Noble et al. (2017b)
Meclonazepam	U	(ARS/GRD)-DIL	LC-QTOF-MS	Auto-MS/MS	Metabolism studies	Vikingsson et al. (2017)
Clonazolam, meclonazepam, nifoxipam	U	DIL	(Nano)LC-OT-MS/MS	Full scan and TMS^2	Comparison LC-OT-MS/MS to (nano) LC-OT-MS/MS, targets recommended for urine drug testing	Meyer et al. (2016)
Flubromazolam, pyrazolam	U	DIL(-GRD)	LC-OT-MS/MS	Full scan or PRM	Targets recommended for urine drug testing	Pettersson Bergstrand et al. (2017b)
11 designer benzodiazepines	U	DIL-GRD	LC-MS/MS	SRM	Multicomponent method for detection and quantification	Pettersson Bergstrand et al. (2016)

ARS arylsulfatase, *B* whole blood, *$ddMS^2$* data-dependent MS^2, *DIA* data-independent acquisition, *DIL* dilution, *GRD* β-glucuronidase, *H* hair, *LC* liquid chromatography, *LLE* liquid-liquid extraction, *ME* extraction with methanol, *MS/MS* tandem mass spectrometry, *OT* orbitrap, *PRE* precipitation, *PRM* parallel reaction monitoring, *QTOF* quadrupole time-of-flight, *S* serum, *SRM* selected reaction monitoring, *T* tissue, *TMS^2* targeted MS^2, *U* urine

were only capable of producing minor amounts of the amino metabolite (Vikingsson et al. 2017). Meyer et al. were also interested in the identification of main urinary metabolites of three nitrobenzodiazepines in authentic urine samples and compared results obtained by nanoLC-HRMS/MS to conventional ultra-high performance LC-HRMS/MS (Meyer et al. 2016). The nanoLC system was found to provide a higher abundance of all signals and consequently higher sensitivity allowing for detection of additional compounds. Whereas clonazolam and meclonazepam were mainly excreted as their amino and acetamino metabolites, nifoxipam was additionally detected as glucuronide. As the parent compounds were generally present in low concentrations or not found at all, the authors emphasized the need to involve metabolites in the used screening procedure and recommended 7-aminoclonazolam, 7-acetaminomeclonazepam, and 7-acetaminonifoxipam as suitable targets for urine drug testing. Concerning flubromazolam and pyrazolam, recommended targets for urine screening were described by Pettersson Bergstrand et al. (2017b). The same authors published a multicomponent LC-MS/MS method for determination and quantification of 11 designer benzodiazepines in urine after conjugate cleavage. The method was applied to analysis of 390 samples with a positive immunoassay benzodiazepine screening but a negative MS confirmation focusing on prescribed benzodiazepines. They could detect designer benzodiazepines in 40% of these cases (Pettersson Bergstrand et al. 2016).

9 Bioanalysis of Hallucinogenic NPS

Hallucinogens are a diverse group of drugs that alter perception, thoughts, and feelings. Naturally occurring compounds, such as tryptamines, produced by plants, mushrooms, or animals, as well as human-made hallucinogens such as lysergic acid diethylamide (LSD), are used (NIDA 2016). Synthetic tryptamines and LSD derivatives are available on the drugs of abuse market. Arylcyclohexamines, which include derivatives of ketamine or phencyclidine, and 2-methoxybenzyl-substituted amines, the so-called NBOMes (Kyriakou et al. 2015; Zawilska 2014), are also included in this particular group of compounds. Chemically, NBOMes are derivatives of phenethylamine, but examples of amphetamine- and tryptamine-based NBOMes have also been characterized (Brandt et al. 2015; Caspar et al. 2017c).

The review article published by Kyriakou et al. also contained analytical methods for the detection of NBOMes (Kyriakou et al. 2015). Experimental setups and highlights of original research articles containing analytical methods for detection of hallucinogens are summarized in Table 6. Since phenethylamine and lysergamide-based NPS are rather low-dosed, their reliable detection and identification in biosamples may be difficult. Caspar et al. published the simultaneous identification and quantification of low-dosed hallucinogens in blood plasma based on LC-HRMS including three LSD derivatives and five NBOMes (Caspar et al. 2018). Poklis et al. published a detection method for nine different NBOMes in urine specimens, applied it to authentic samples, and identified four different NBOMes

Table 6 Biosamples, experimental setups, and highlights of studies containing analytical methods for detection of hallucinogens

NPS	Biosample	Workup	Instrumentation	Detection mode	Highlights	Reference
AL-LAD, LSZ, 1P-LSD, 25B-, 25C-, 25E-, 25H-, and 25I-NBOMe	P	LLE	LC-OT-MS/MS	Full scan and AIF	Simultaneous detection and quantification, validation criteria acc. to the European Medicines Agency fulfilled for LSD derivatives	Caspar et al. (2018)
9 NBOMes	U	SPE	LC-MS/MS	SRM	Broad range of NBOMes	Poklis et al. (2014)
25B-NBOMe	P, U	Not given	LC-MS/MS	SRM	Plasma and urine concentrations	Gee et al. (2016)
Methoxetamine	U	DIL	LC-OT-MS/MS	Full scan	Phase I and II metabolism study	Menzies et al. (2014)
3-MeO-PCP, 4-MeO-PCP	S, U	PRE, DIL	LC-MS/MS, LC-HRMS/MS	SRM, full scan	NPS concentrations, cross-reactivity in a PCP immunoassay only for high concentrations	Backberg et al. (2015a)
5-IT	S, U	PRE, DIL	LC-MS/MS	SRM	NPS concentrations and comparison between nonfatal and fatal cases	Backberg et al. (2014)

AIF all-ions fragmentation, *DIL* dilution, *HRMS/MS* high-resolution tandem mass spectrometry, *LC* liquid chromatography, *LLE* liquid-liquid extraction, *MS/MS* tandem mass spectrometry, *OT* orbitrap, *P* plasma, *PRE* precipitation, *S* serum, *SPE* solid-phase extraction, *SRM* selected reaction monitoring, *U* urine

(Poklis et al. 2014). Unfortunately, no metabolites were included, even though extensive information on metabolism of NBOMes have been described (Caspar et al. 2015, 2017; Temporal et al. 2017; Wohlfarth et al. 2017). Gee et al. described an LC-MS/MS method for determination of plasma and urine concentrations of 25B-NBOMe in a series of intoxication cases, although the use of 25D-NBOMe as an internal standard could be problematic in cases of polydrug abuse (Gee et al. 2016).

The use of arylcyclohexamines among NPS users was also confirmed by two case series. Menzies et al. investigated the metabolic fate of the ketamine analog methoxetamine using in vitro incubations and authentic urine samples (Menzies et al. 2014). Backberg et al. reported 67 intoxication cases with phencyclidine derivatives that occurred in Sweden between July 2013 and March 2015 (Backberg et al. 2015a). For synthetic tryptamines, only few studies were published, focused mainly on metabolism (Caspar et al. 2017b; Grafinger et al. 2017; Michely et al. 2015, 2017). However, a case series from the STRIDA project describing intoxications with 5-(2-aminopropyl)indole (5-IT), a positional isomer of alpha-methyltryptamine (AMT, 3-IT), was published (Backberg et al. 2014). Similar to AMT, 5-IT was shown to be a reversible inhibitor of monoamine oxidase A and is difficult to be analytically differentiated from AMT (Elliott et al. 2013; Shulgin and Shulgin 1997; Wagmann et al. 2017). For this reason, 5-IT is mentioned here although being a stimulant (Shulgin and Shulgin 1997). Furthermore, hallucinogens, mainly tryptamines and NBOMes, but also some arylcyclohexamines, were included in the multi-analyte approaches discussed before (Adamowicz and Tokarczyk 2016; Al-Saffar et al. 2013; Ambach et al. 2014; Boumba et al. 2017; Lee et al. 2016; Lehmann et al. 2017; Montesano et al. 2017; Stephanson et al. 2017; Tang et al. 2014; Vaiano et al. 2016).

10 Conclusions

The ever-changing pool of NPS flooding the drugs of abuse market is certainly challenging for clinical and forensic toxicology. Nevertheless, the high number of research articles describing bioanalytical methods for detection of NPS published during the last 5 years demonstrated that this problem is widely known these days. While immunoassays were found to be rather inappropriate for detection of NPS, MS-based procedures proved to be suitable due to high flexibility, sensitivity, and selectivity. Especially HR devices are promising tools for identification of unknown compounds or very low-dosed substances. However, in comparison to low-resolution devices, these instruments are significantly more expensive, and data handling is more sophisticated. Nevertheless, their application will further increase during the next years if these disadvantages will be overcome.

References

Adamowicz P, Tokarczyk B (2016) Simple and rapid screening procedure for 143 new psychoactive substances by liquid chromatography-tandem mass spectrometry. Drug Test Anal 8:652–667

Adamowicz P, Gieron J, Gil D, Lechowicz W, Skulska A, Tokarczyk B (2016) 3-Methylmethcathinone-interpretation of blood concentrations based on analysis of 95 cases. J Anal Toxicol 40:272–276

Adams AJ, Banister SD, Irizarry L, Trecki J, Schwartz M, Gerona R (2017) "Zombie" outbreak caused by the synthetic cannabinoid AMB-FUBINACA in New York. N Engl J Med 376:235–242

Al-Saffar Y, Stephanson NN, Beck O (2013) Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine-experience from the Swedish population. J Chromatogr B Analyt Technol Biomed Life Sci 930:112–120

Alvarez JC, Fabresse N, Larabi IA (2017) Prevalence and surveillance of synthetic cathinones use by hair analysis: an update review. Curr Pharm Des. https://doi.org/10.2174/1381612823666170704124156

Ambach L, Hernandez Redondo A, Konig S, Weinmann W (2014) Rapid and simple LC-MS/MS screening of 64 novel psychoactive substances using dried blood spots. Drug Test Anal 6:367–375

Ares AM, Fernandez P, Regenjo M, Fernandez AM, Carro AM, Lorenzo RA (2017) A fast bioanalytical method based on microextraction by packed sorbent and UPLC-MS/MS for determining new psychoactive substances in oral fluid. Talanta 174:454–461

Arntson A, Ofsa B, Lancaster D, Simon JR, McMullin M, Logan B (2013) Validation of a novel immunoassay for the detection of synthetic cannabinoids and metabolites in urine specimens. J Anal Toxicol 37:284–290

Backberg M, Beck O, Hulten P, Rosengren-Holmberg J, Helander A (2014) Intoxications of the new psychoactive substance 5-(2-aminopropyl)indole (5-IT): a case series from the Swedish STRIDA project. Clin Toxicol (Phila) 52:618–624

Backberg M, Beck O, Helander A (2015a) Phencyclidine analog use in Sweden – intoxication cases involving 3-MeO-PCP and 4-MeO-PCP from the STRIDA project. Clin Toxicol (Phila) 53:856–864

Backberg M, Beck O, Jonsson KH, Helander A (2015b) Opioid intoxications involving butyrfentanyl, 4-fluorobutyrfentanyl, and fentanyl from the Swedish STRIDA project. Clin Toxicol (Phila) 53:609–617

Backberg M, Lindeman E, Beck O, Helander A (2015c) Characteristics of analytically confirmed 3-MMC-related intoxications from the Swedish STRIDA project. Clin Toxicol (Phila) 53:46–53

Backberg M, Westerbergh J, Beck O, Helander A (2016) Adverse events related to the new psychoactive substance 3-fluorophenmetrazine – results from the Swedish STRIDA project. Clin Toxicol (Phila) 54:819–825

Backberg M, Tworek L, Beck O, Helander A (2017) Analytically confirmed intoxications involving MDMB-CHMICA from the STRIDA project. J Med Toxicol 13:52–60

Barnes AJ, Spinelli E, Young S, Martin TM, Kleete KL, Huestis MA (2015) Validation of an ELISA synthetic cannabinoids urine assay. Ther Drug Monit 37:661–669

Beck O, Rausberg L, Al-Saffar Y, Villen T, Karlsson L, Hansson T, Helander A (2014) Detectability of new psychoactive substances, 'legal highs', in CEDIA, EMIT, and KIMS immunochemical screening assays for drugs of abuse. Drug Test Anal 6:492–499

Beck O, Franzen L, Backberg M, Signell P, Helander A (2015) Intoxications involving MDPV in Sweden during 2010-2014: results from the STRIDA project. Clin Toxicol (Phila) 53:865–873

Beck O, Franzen L, Backberg M, Signell P, Helander A (2016) Toxicity evaluation of alpha-pyrrolidinovalerophenone (alpha-PVP): results from intoxication cases within the STRIDA project. Clin Toxicol (Phila) 54:568–575

Beck O, Backberg M, Signell P, Helander A (2017) Intoxications in the STRIDA project involving a panorama of psychostimulant pyrovalerone derivatives, MDPV copycats. Clin Toxicol (Phila) 12:1–8

Borg D, Tverdovsky A, Stripp R (2017) A fast and comprehensive analysis of 32 synthetic cannabinoids using agilent triple quadrupole LC-MS-MS. J Anal Toxicol 41:6–16

Boumba VA, Di Rago M, Peka M, Drummer OH, Gerostamoulos D (2017) The analysis of 132 novel psychoactive substances in human hair using a single step extraction by tandem LC/MS. Forensic Sci Int 279:192–202

Brandt SD, Elliott SP, Kavanagh PV, Dempster NM, Meyer MR, Maurer HH, Nichols DE (2015) Analytical characterization of bioactive N-benzyl-substituted phenethylamines and 5-methoxytryptamines. Rapid Commun Mass Spectrom 29:573–584

Cannaert A, Storme J, Franz F, Auwarter V, Stove CP (2016) Detection and activity profiling of synthetic cannabinoids and their metabolites with a newly developed bioassay. Anal Chem 88:11476–11485

Cannaert A, Franz F, Auwarter V, Stove CP (2017) Activity-based detection of consumption of synthetic cannabinoids in authentic urine samples using a stable cannabinoid reporter system. Anal Chem 89:9527–9536

Caspar AT, Helfer AG, Michely JA, Auwarter V, Brandt SD, Meyer MR, Maurer HH (2015) Studies on the metabolism and toxicological detection of the new psychoactive designer drug 2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOMe) in human and rat urine using GC-MS, LC-MS(n), and LC-HR-MS/MS. Anal Bioanal Chem 407:6697–6719

Caspar AT, Brandt SD, Stoever AE, Meyer MR, Maurer HH (2017a) Metabolic fate and detectability of the new psychoactive substances 2-(4-bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl] ethanamine (25B-NBOMe) and 2-(4-chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl] ethanamine (25C-NBOMe) in human and rat urine by GC-MS, LC-MSn, and LC-HR-MS/MS approaches. J Pharm Biomed Anal 134:158–169

Caspar AT, Gaab JB, Michely JA, Brandt SD, Meyer MR, Maurer HH (2017b) Metabolism of the tryptamine-derived new psychoactive substances 5-MeO-2-Me-DALT, 5-MeO-2-Me-ALCHT, and 5-MeO-2-Me-DIPT and their detectability in urine studied by GC-MS, LC-MSn, and LC-HR-MS/MS. Drug Test Anal. https://doi.org/10.1002/dta.2197

Caspar AT, Westphal F, Meyer MR, Maurer HH (2017c) LC-high resolution-MS/MS for identification of 69 metabolites of the new psychoactive substance 1-(4-ethylphenyl-)-N-[(2-methoxyphenyl)methyl] propane-2-amine (4-EA-NBOMe) in rat urine and human liver S9 incubates and comparison of its screening power with further MS techniques. Anal Bioanal Chem. https://doi.org/10.1007/s00216-017-0526-0

Caspar AT, Kollas AB, Maurer HH, Meyer MR (2018) Development of a quantitative approach in blood plasma for low-dosed hallucinogens and opioids using LC-high resolution mass spectrometry. Talanta 176:635–645

Castaneto MS, Barnes AJ, Concheiro M, Klette KL, Martin TA, Huestis MA (2015a) Biochip array technology immunoassay performance and quantitative confirmation of designer piperazines for urine workplace drug testing. Anal Bioanal Chem 407:4639–4648

Castaneto MS, Wohlfarth A, Desrosiers NA, Hartman RL, Gorelick DA, Huestis MA (2015b) Synthetic cannabinoids pharmacokinetics and detection methods in biological matrices. Drug Metab Rev 47:124–174

Concheiro M, Castaneto M, Kronstrand R, Huestis MA (2015) Simultaneous determination of 40 novel psychoactive stimulants in urine by liquid chromatography-high resolution mass spectrometry and library matching. J Chromatogr A 1397:32–42

Deluca P, Davey Z, Corazza O, Di Furia L, Farre M, Flesland LH, Mannonen M, Majava A, Peltoniemi T, Pasinetti M, Pezzolesi C, Scherbaum N, Siemann H, Skutle A, Torrens M, van der Kreeft P, Iversen E, Schifano F (2012) Identifying emerging trends in recreational drug use; outcomes from the Psychonaut Web Mapping Project. Prog Neuro-Psychopharmacol Biol Psychiatry 39:221–226

Ellefsen KN, Concheiro M, Huestis MA (2016) Synthetic cathinone pharmacokinetics, analytical methods, and toxicological findings from human performance and postmortem cases. Drug Metab Rev 48:237–265

Elliott SP, Brandt SD, Freeman S, Archer RP (2013) AMT (3-(2-aminopropyl)indole) and 5-IT (5-(2-aminopropyl)indole): an analytical challenge and implications for forensic analysis. Drug Test Anal 5:196–202

EMCDDA (2017) European drug report 2017: trends and developments. European Monitoring Centre for Drugs and Drug Addiction. http://www.emcdda.europa.eu/system/files/publications/4541/TDAT17001ENN.pdf. Accessed 11 Dec 2017

Fantegrossi WE, Moran JH, Radominska-Pandya A, Prather PL (2014) Distinct pharmacology and metabolism of K2 synthetic cannabinoids compared to delta(9)-THC: mechanism underlying greater toxicity? Life Sci 97:45–54

Favretto D, Pascali JP, Tagliaro F (2013) New challenges and innovation in forensic toxicology: focus on the "new psychoactive substances". J Chromatogr A 1287:84–95

Fleming SW, Cooley JC, Johnson L, Frazee CC, Domanski K, Kleinschmidt K, Garg U (2017) Analysis of U-47700, a novel synthetic opioid, in human urine by LC-MS-MS and LC-QToF. J Anal Toxicol 41:173–180

Franz F, Angerer V, Jechle H, Pegoro M, Ertl H, Weinfurtner G, Janele D, Schlogl C, Friedl M, Gerl S, Mielke R, Zehnle R, Wagner M, Moosmann B, Auwarter V (2017a) Immunoassay screening in urine for synthetic cannabinoids – an evaluation of the diagnostic efficiency. Clin Chem Lab Med 55:1375–1384

Franz F, Angerer V, Moosmann B, Auwarter V (2017b) Phase I metabolism of the highly potent synthetic cannabinoid MDMB-CHMICA and detection in human urine samples. Drug Test Anal 9:744–753

Gee P, Schep LJ, Jensen BP, Moore G, Barrington S (2016) Case series: toxicity from 25B-NBOMe – a cluster of N-bomb cases. Clin Toxicol (Phila) 54:141–146

Grafinger KE, Hadener M, Konig S, Weinmann W (2017) Study of the in vitro and in vivo metabolism of the tryptamine 5-MeO-MiPT using human liver microsomes and real case samples. Drug Test Anal. https://doi.org/10.1002/dta.2245

Grapp M, Kaufmann C, Ebbecke M (2017) Toxicological investigation of forensic cases related to the designer drug 3,4-methylenedioxypyrovalerone (MDPV): detection, quantification and studies on human metabolism by GC-MS. Forensic Sci Int 273:1–9

Helander A, Backberg M, Beck O (2014a) MT-45, a new psychoactive substance associated with hearing loss and unconsciousness. Clin Toxicol (Phila) 52:901–904

Helander A, Backberg M, Hulten P, Al-Saffar Y, Beck O (2014b) Detection of new psychoactive substance use among emergency room patients: results from the Swedish STRIDA project. Forensic Sci Int 243:23–29

Helander A, Backberg M, Beck O (2016) Intoxications involving the fentanyl analogs acetylfentanyl, 4-methoxybutyrfentanyl and furanylfentanyl: results from the Swedish STRIDA project. Clin Toxicol (Phila) 54:324–332

Helander A, Backberg M, Signell P, Beck O (2017) Intoxications involving acrylfentanyl and other novel designer fentanyls – results from the Swedish STRIDA project. Clin Toxicol (Phila) 55:589–599

Helfer AG, Michely JA, Weber AA, Meyer MR, Maurer HH (2015) Orbitrap technology for comprehensive metabolite-based liquid chromatographic-high resolution-tandem mass spectrometric urine drug screening – exemplified for cardiovascular drugs. Anal Chim Acta 891:221–233

Hoiseth G, Tuv SS, Karinen R (2016) Blood concentrations of new designer benzodiazepines in forensic cases. Forensic Sci Int 268:35–38

Huppertz LM, Moosmann B, Auwarter V (2017) Flubromazolam – basic pharmacokinetic evaluation of a highly potent designer benzodiazepine. Drug Test Anal. https://doi.org/10.1002/dta.2203

Karila L, Benyamina A, Blecha L, Cottencin O, Billieux J (2016) The synthetic cannabinoids phenomenon. Curr Pharm Des 22:6420–6425

Karinen R, Tuv SS, Oiestad EL, Vindenes V (2015) Concentrations of APINACA, 5F-APINACA, UR-144 and its degradant product in blood samples from six impaired drivers compared to previous reported concentrations of other synthetic cannabinoids. Forensic Sci Int 246:98–103

Kyriakou C, Marinelli E, Frati P, Santurro A, Afxentiou M, Zaami S, Busardo FP (2015) NBOMe: new potent hallucinogens-pharmacology, analytical methods, toxicities, fatalities: a review. Eur Rev Med Pharmacol Sci 19:3270–3281

Lee HH, Lee JF, Lin SY, Chen BH (2016) Simultaneous identification of abused drugs, benzodiazepines, and new psychoactive substances in urine by liquid chromatography tandem mass spectrometry. Kaohsiung J Med Sci 32:118–127

Lehmann S, Kieliba T, Beike J, Thevis M, Mercer-Chalmers-Bender K (2017) Determination of 74 new psychoactive substances in serum using automated in-line solid-phase extraction-liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 1064:124–138

Lendoiro E, Jimenez-Morigosa C, Cruz A, Paramo M, Lopez-Rivadulla M, de Castro A (2017) An LC-MS/MS methodological approach to the analysis of hair for amphetamine-type-stimulant (ATS) drugs, including selected synthetic cathinones and piperazines. Drug Test Anal 9:96–105

Logan BK, Mohr ALA, Friscia M, Krotulski AJ, Papsun DM, Kacinko SL, Ropero-Miller JD, Huestis MA (2017) Reports of adverse events associated with use of novel psychoactive substances, 2013-2016: a review. J Anal Toxicol 41:573–610

Maas A, Sydow K, Madea B, Hess C (2017) Separation of ortho, meta and para isomers of methylmethcathinone (MMC) and methylethcathinone (MEC) using LC-ESI-MS/MS: application to forensic serum samples. J Chromatogr B Analyt Technol Biomed Life Sci 1051:118–125

Manchester KR, Lomas EC, Waters L, Dempsey FC, Maskell PD (2017) The emergence of new psychoactive substance (NPS) benzodiazepines: a review. Drug Test Anal. https://doi.org/10.1002/dta.2211

Maurer HH, Meyer MR (2016) High-resolution mass spectrometry in toxicology: current status and future perspectives. Arch Toxicol 90:2161–2172

Menzies EL, Hudson SC, Dargan PI, Parkin MC, Wood DM, Kicman AT (2014) Characterizing metabolites and potential metabolic pathways for the novel psychoactive substance methoxetamine. Drug Test Anal 6:506–515

Mercolini L, Protti M (2016) Biosampling strategies for emerging drugs of abuse: towards the future of toxicological and forensic analysis. J Pharm Biomed Anal 130:202–219

Meyer MR (2016) New psychoactive substances: an overview on recent publications on their toxicodynamics and toxicokinetics. Arch Toxicol 90:2421–2444

Meyer MR, Maurer HH (2016) Review: LC coupled to low- and high-resolution mass spectrometry for new psychoactive substance screening in biological matrices – where do we stand today? Anal Chim Acta 927:13–20

Meyer MR, Bergstrand MP, Helander A, Beck O (2016) Identification of main human urinary metabolites of the designer nitrobenzodiazepines clonazolam, meclonazepam, and nifoxipam by nano-liquid chromatography-high-resolution mass spectrometry for drug testing purposes. Anal Bioanal Chem 408:3571–3591

Michely JA, Helfer AG, Brandt SD, Meyer MR, Maurer HH (2015) Metabolism of the new psychoactive substances N,N-diallyltryptamine (DALT) and 5-methoxy-DALT and their detectability in urine by GC-MS, LC-MSn, and LC-HR-MS-MS. Anal Bioanal Chem 407:7831–7842

Michely JA, Brandt SD, Meyer MR, Maurer HH (2017) Biotransformation and detectability of the new psychoactive substances N,N-diallyltryptamine (DALT) derivatives 5-fluoro-DALT, 7-methyl-DALT, and 5,6-methylenedioxy-DALT in urine using GC-MS, LC-MSn, and LC-HR-MS/MS. Anal Bioanal Chem 409:1681–1695

Mogler L, Franz F, Rentsch D, Angerer V, Weinfurtner G, Longworth M, Banister SD, Kassiou M, Moosmann B, Auwarter V (2017) Detection of the recently emerged synthetic cannabinoid 5F-MDMB-PICA in 'legal high' products and human urine samples. Drug Test Anal. https://doi.org/10.1002/dta.2201

Mohr AL, Ofsa B, Keil AM, Simon JR, McMullin M, Logan BK (2014) Enzyme-linked immunosorbent assay (ELISA) for the detection of use of the synthetic cannabinoid agonists UR-144 and XLR-11 in human urine. J Anal Toxicol 38:427–431

Mollerup CB, Dalsgaard PW, Mardal M, Linnet K (2017) Targeted and non-targeted drug screening in whole blood by UHPLC-TOF-MS with data-independent acquisition. Drug Test Anal 9:1052–1061

Montesano C, Vannutelli G, Gregori A, Ripani L, Compagnone D, Curini R, Sergi M (2016) Broad screening and identification of novel psychoactive substances in plasma by high-performance liquid chromatography-high-resolution mass spectrometry and post-run library matching. J Anal Toxicol 40:519–528

Montesano C, Vannutelli G, Massa M, Simeoni MC, Gregori A, Ripani L, Compagnone D, Curini R, Sergi M (2017) Multi-class analysis of new psychoactive substances and metabolites in hair by pressurized liquid extraction coupled to HPLC-HRMS. Drug Test Anal 9:798–807

Moosmann B, Huppertz LM, Hutter M, Buchwald A, Ferlaino S, Auwarter V (2013) Detection and identification of the designer benzodiazepine flubromazepam and preliminary data on its metabolism and pharmacokinetics. J Mass Spectrom 48:1150–1159

Moosmann B, Bisel P, Auwarter V (2014) Characterization of the designer benzodiazepine diclazepam and preliminary data on its metabolism and pharmacokinetics. Drug Test Anal 6:757–763

Moosmann B, Bisel P, Franz F, Huppertz LM, Auwarter V (2016) Characterization and in vitro phase I microsomal metabolism of designer benzodiazepines – an update comprising adinazolam, cloniprazepam, fonazepam, 3-hydroxyphenazepam, metizolam, and nitrazolam. J Mass Spectrom 51:1080–1089

Nelson ME, Bryant SM, Aks SE (2014) Emerging drugs of abuse. Emerg Med Clin North Am 32:1–28

NIDA (2016) Hallucinogens. https://www.drugabuse.gov/publications/drugfacts/hallucinogens. Accessed 11 Dec 2017

Nieddu M, Burrai L, Baralla E, Pasciu V, Varoni MV, Briguglio I, Demontis MP, Boatto G (2016) ELISA detection of 30 new amphetamine designer drugs in whole blood, urine and oral fluid using Neogen® "amphetamine" and "methamphetamine/MDMA" kits. J Anal Toxicol 40:492–497

Noble C, Dalsgaard PW, Johansen SS, Linnet K (2017a) Application of a screening method for fentanyl and its analogues using UHPLC-QTOF-MS with data-independent acquisition (DIA) in MSE mode and retrospective analysis of authentic forensic blood samples. Drug Test Anal. https://doi.org/10.1002/dta.2263

Noble C, Mardal M, Bjerre Holm N, Stybe Johansen S, Linnet K (2017b) In vitro studies on flubromazolam metabolism and detection of its metabolites in authentic forensic samples. Drug Test Anal 9:1182–1191

O'Connor LC, Torrance HJ, McKeown DA (2016) ELISA detection of phenazepam, etizolam, pyrazolam, flubromazepam, diclazepam and delorazepam in blood using Immunalysis® benzodiazepine kit. J Anal Toxicol 40:159–161

Odoardi S, Fisichella M, Romolo FS, Strano-Rossi S (2015) High-throughput screening for new psychoactive substances (NPS) in whole blood by DLLME extraction and UHPLC-MS/MS analysis. J Chromatogr B Analyt Technol Biomed Life Sci 1000:57–68

Olesti E, Pujadas M, Papaseit E, Perez-Mana C, Pozo OJ, Farre M, de la Torre R (2017) GC-MS quantification method for mephedrone in plasma and urine: application to human pharmacokinetics. J Anal Toxicol 41:100–106

Papsun D, Krywanczyk A, Vose JC, Bundock EA, Logan BK (2016) Analysis of MT-45, a novel synthetic opioid, in human whole blood by LC-MS-MS and its identification in a drug-related death. J Anal Toxicol 40:313–317

Peters FT (2014) Recent developments in urinalysis of metabolites of new psychoactive substances using LC-MS. Bioanalysis 6:2083–2107

Pettersson Bergstrand M, Helander A, Beck O (2016) Development and application of a multicomponent LC-MS/MS method for determination of designer benzodiazepines in urine. J Chromatogr B Analyt Technol Biomed Life Sci 1035:104–110

Pettersson Bergstrand M, Helander A, Hansson T, Beck O (2017a) Detectability of designer benzodiazepines in CEDIA, EMIT II Plus, HEIA, and KIMS II immunochemical screening assays. Drug Test Anal 9:640–645

Pettersson Bergstrand M, Meyer MR, Beck O, Helander A (2017b) Human urinary metabolic patterns of the designer benzodiazepines flubromazolam and pyrazolam studied by liquid chromatography-high resolution mass spectrometry. Drug Test Anal. https://doi.org/10.1002/dta.2243

Poklis JL, Clay DJ, Poklis A (2014) High-performance liquid chromatography with tandem mass spectrometry for the determination of nine hallucinogenic 25-NBOMe designer drugs in urine specimens. J Anal Toxicol 38:113–121

Protti M, Rudge J, Sberna AE, Gerra G, Mercolini L (2017) Dried haematic microsamples and LC-MS/MS for the analysis of natural and synthetic cannabinoids. J Chromatogr B Analyt Technol Biomed Life Sci 1044–1045:77–86

Richter LHJ, Maurer HH, Meyer MR (2017) New psychoactive substances: studies on the metabolism of XLR-11, AB-PINACA, FUB-PB-22, 4-methoxy-alpha-PVP, 25-I-NBOMe, and meclonazepam using human liver preparations in comparison to primary human hepatocytes, and human urine. Toxicol Lett 280:142–150

Salomone A, Luciano C, Di Corcia D, Gerace E, Vincenti M (2014) Hair analysis as a tool to evaluate the prevalence of synthetic cannabinoids in different populations of drug consumers. Drug Test Anal 6:126–134

Salomone A, Gazzilli G, Di Corcia D, Gerace E, Vincenti M (2016) Determination of cathinones and other stimulant, psychedelic, and dissociative designer drugs in real hair samples. Anal Bioanal Chem 408:2035–2042

Schaefer N, Wojtyniak JG, Kettner M, Schlote J, Laschke MW, Ewald AH, Lehr T, Menger MD, Maurer HH, Schmidt PH (2016) Pharmacokinetics of (synthetic) cannabinoids in pigs and their relevance for clinical and forensic toxicology. Toxicol Lett 253:7–16

Shulgin A, Shulgin A (1997) Tihkal: the continuation. Transform Press, Berkley

Stephanson NN, Signell P, Helander A, Beck O (2017) Use of LC-HRMS in full scan-XIC mode for multi-analyte urine drug testing – a step towards a 'black-box' solution? J Mass Spectrom 52:497–506

Steuer AE, Williner E, Staeheli SN, Kraemer T (2017) Studies on the metabolism of the fentanyl-derived designer drug butyrfentanyl in human in vitro liver preparations and authentic human samples using liquid chromatography-high resolution mass spectrometry (LC-HRMS). Drug Test Anal 9:1085–1092

Strano-Rossi S, Odoardi S, Fisichella M, Anzillotti L, Gottardo R, Tagliaro F (2014) Screening for new psychoactive substances in hair by ultrahigh performance liquid chromatography-electrospray ionization tandem mass spectrometry. J Chromatogr A 1372C:145–156

Sundstrom M, Pelander A, Ojanpera I (2015) Comparison between drug screening by immunoassay and ultra-high performance liquid chromatography/high-resolution time-of-flight mass spectrometry in post-mortem urine. Drug Test Anal 7:420–427

Sundstrom M, Pelander A, Ojanpera I (2017) Comparison of post-targeted and pre-targeted urine drug screening by UHPLC-HR-QTOFMS. J Anal Toxicol 41:623–630

SWGDRUG (2016) Recommendations for the analysis of seized drugs. http://www.swgdrug.org/Documents/SWGDRUG%20Recommendations%20Version%207-0.pdf. Accessed 11 Dec 2017

Swortwood MJ, Hearn WL, DeCaprio AP (2014) Cross-reactivity of designer drugs, including cathinone derivatives, in commercial enzyme-linked immunosorbent assays. Drug Test Anal 6:716–727

Swortwood MJ, Carlier J, Ellefsen KN, Wohlfarth A, Diao X, Concheiro-Guisan M, Kronstrand R, Huestis MA (2016a) In vitro, in vivo and in silico metabolic profiling of alpha-pyrrolidinopentiothiophenone, a novel thiophene stimulant. Bioanalysis 8:65–82

Swortwood MJ, Ellefsen KN, Wohlfarth A, Diao X, Concheiro-Guisan M, Kronstrand R, Huestis MA (2016b) First metabolic profile of PV8, a novel synthetic cathinone, in human hepatocytes and urine by high-resolution mass spectrometry. Anal Bioanal Chem 408:4845–4856

Tang MH, Ching CK, Lee CY, Lam YH, Mak TW (2014) Simultaneous detection of 93 conventional and emerging drugs of abuse and their metabolites in urine by UHPLC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 969:272–284

Temporal KH, Scott KS, Mohr ALA, Logan BK (2017) Metabolic profile determination of NBOMe compounds using human liver microsomes and comparison with findings in authentic human blood and urine. J Anal Toxicol 41:646–657

Tynon M, Homan J, Kacinko S, Ervin A, McMullin M, Logan BK (2017) Rapid and sensitive screening and confirmation of thirty-four aminocarbonyl/carboxamide (NACA) and arylindole synthetic cannabinoid drugs in human whole blood. Drug Test Anal 9:924–934

Tyrkko E, Andersson M, Kronstrand R (2016) The toxicology of new psychoactive substances: synthetic cathinones and phenylethylamines. Ther Drug Monit 38:190–216

Vaiano F, Busardo FP, Palumbo D, Kyriakou C, Fioravanti A, Catalani V, Mari F, Bertol E (2016) A novel screening method for 64 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases. J Pharm Biomed Anal 129:441–449

Vikingsson S, Green H, Brinkhagen L, Mukhtar S, Josefsson M (2016) Identification of AB-FUBINACA metabolites in authentic urine samples suitable as urinary markers of drug intake using liquid chromatography quadrupole tandem time of flight mass spectrometry. Drug Test Anal 8:950–956

Vikingsson S, Wohlfarth A, Andersson M, Green H, Roman M, Josefsson M, Kugelberg FC, Kronstrand R (2017) Identifying metabolites of meclonazepam by high-resolution mass spectrometry using human liver microsomes, hepatocytes, a mouse model, and authentic urine samples. AAPS J 19:736–742

Wagmann L, Brandt SD, Kavanagh PV, Maurer HH, Meyer MR (2017) In vitro monoamine oxidase inhibition potential of alpha-methyltryptamine analog new psychoactive substances for assessing possible toxic risks. Toxicol Lett 272:84–93

Watanabe S, Vikingsson S, Roman M, Green H, Kronstrand R, Wohlfarth A (2017) In vitro and in vivo metabolite identification studies for the new synthetic opioids acetylfentanyl, acrylfentanyl, furanylfentanyl, and 4-fluoro-isobutyrylfentanyl. AAPS J 19:1102–1122

Welter-Luedeke J, Maurer HH (2016) New psychoactive substances: chemistry, pharmacology, metabolism, and detectability of amphetamine derivatives with modified ring systems. Ther Drug Monit 38:4–11

Williams M, Martin J, Galettis P (2017) A validated method for the detection of 32 bath salts in oral fluid. J Anal Toxicol 41:659–669

Wissenbach DK, Meyer MR, Remane D, Weber AA, Maurer HH (2011) Development of the first metabolite-based LC-MS(n) urine drug screening procedure-exemplified for antidepressants. Anal Bioanal Chem 400:79–88

Wohlfarth A, Roman M, Andersson M, Kugelberg FC, Diao X, Carlier J, Eriksson C, Wu X, Konradsson P, Josefsson M, Huestis MA, Kronstrand R (2017) 25C-NBOMe and 25I-NBOMe metabolite studies in human hepatocytes, in vivo mouse and human urine with high-resolution mass spectrometry. Drug Test Anal 9:680–698

Zamengo L, Frison G, Bettin C, Sciarrone R (2014) Understanding the risks associated with the use of new psychoactive substances (NPS): high variability of active ingredients concentration, mislabelled preparations, multiple psychoactive substances in single products. Toxicol Lett 229:220–228

Zawilska JB (2014) Methoxetamine-a novel recreational drug with potent hallucinogenic properties. Toxicol Lett 230:402–407

Toxicokinetics of NPS: Update 2017

Markus R. Meyer

Contents

Abstract

This summarizing and descriptive review article is an update on previously published reviews. It covers English-written and PubMed-listed review articles and original studies published between May 2016 and November 2017 on the toxicokinetics of new psychoactive substances (NPS). Compounds covered include stimulants and entactogens, synthetic cannabinoids, tryptamines, phenethylamine and phencyclidine-like drugs, benzodiazepines, and opioids. First, an overview and discussion is provided on selected review articles followed by an overview and discussion on selected original studies. Both sections are then concluded by an opinion on these latest developments. The present review shows that the NPS market is still highly dynamic and that studies regarding their toxicokinetics are necessary to understand risks associated with their consumption. Data collection and studies are encouraged to allow for detection of NPS

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in biological matrices in cases of acute intoxications or chronic consumption. Although some data are available, scientific papers dealing with the mechanistic reasons behind acute and chronic toxicity are still lacking.

Keywords
New psychoactive substances · NPS · Review · Toxicokinetics

Acronyms of the Discussed New Psychoactive Substances (NPS)

25B-NBOMe	2-(4-Bromo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine
25C-NBOMe	2-(4-Bhloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine
25I-NBOH	2-(((4-Iodo-2,5-dimethoxyphenethyl)amino)methyl) phenol
25I-NBOMe	2-(4-Iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine
3-MeO-PCP	3-Methoxyphencyclidine
3-MeO-PCPy	3-Methoxyrolicyclidine
4-FA	4-Fluoroamphetamine
4-methoxy-alpha-PVP	4-Methoxy-alpha-pyrrolidinovalerophenone
4Cl-iBF	4-Chloroisobutyrfentanyl
4F-iBF	4-Fluoroisobutyrfentanyl
5-APB	5-(2-Aminopropyl)benzofuran
5-API	5-(2-Aminopropyl)indole
5-EAPB	5-(2-Ethylaminopropyl)benzofuran
5-MAPB	5-(2-Methylaminopropyl)benzofuran
5-MeO-2-Me-ALCHT	5-Methoxy-2-methyl-*N*-allyl-*N*-cyclohexyltryptamine
5-MeO-2-Me-DALT	5-Methoxy-2-methyl-*N*,*N*-diallyltryptamine
5-MeO-2-Me-DIPT	5-Methoxy-2-methyl-*N*,*N*-diisopropyltryptamine
5-MeO-MIPT	5-Methoxy-*N*-methyl-*N*-isopropyltryptamine
5F-ADB	Methyl (2*S*)-2-{[1-(5-fluoropentyl)-1*H*-indazole-3-carbonyl]amino}-3,3-dimethylbutanoate
5F-CUMYL-P7AICA	1-(5-Fluoropentyl)-*N*-(2-phenylpropan-2-yl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxamide
5F-CUMYL-PINACA	1-(5-Fluoropentyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
5F-MN-18	1-(5-Fluoropentyl)-*N*-(naphthalen-1-yl)-1*H*-indazole-3-carboxamide
7-Me-AMT	7-Methyl-alpha-methyltryptamine
AB-PINACA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-pentyl-1*H*-indazole-3-carboxamide

ADB-FUBINACA	*N*-[(2S)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]-1*H*-indazole-3-carboxamide
AH-7921	3,4-Dichloro-*N*-[(1-dimethylamino)cyclohexylmethyl] benzamide
alpha-PHP	α-Pyrrolidinohexanophenone
alpha-PVP	α-Pyrrolidinovalerophenone
AM-2201	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](naphthalen-1-yl) methanone
AMT	α-Methyltryptamine
cis-4,4′-DMAR	*cis*-4,4′-Dimethylaminorex
CUMYL-4CN-B7AICA	1-(4-Cyanobutyl)-*N*-(2-phenylpropan-2-yl)-1*H*-pyrrolo [2,3-*b*]pyridine-3-carboxamide
CUMYL-4CN-BINACA	1-(4-Cyanobutyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
CUMYL-PINACA	1-Pentyl-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
DALT	*N*,*N*-Diallyltryptamine
FUB-PB-22	Quinolin-8-yl 1-[(4-fluorophenyl)methyl]-1*H*-indole-3-carboxylate
JWH-018	(Naphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone
mCPP	1-(3-Chlorophenyl)piperazine
MDBD	2-Methylamino-1-(3,4-methyledioxyphenyl)butane
MDMA	3,4-Methylenedioxymethamphetamine
MDMB-CHMICA	Methyl (2*S*)-2-{[1-(cyclohexylmethyl)-1*H*-indole-3-carbonyl]amino}-3,3-dimethylbutanoate
MDBP	1-(3,4-Methylenedioxybenzyl)piperazine
MDPPP	3,4-Methylenedioxy-alpha-pyrrolidinopropiophenone
MDPV	3,4-Methylenedioxypyrovalerone
MN-18	*N*-(Naphthalen-1-yl)-1-pentyl-1*H*-indazole-3-carboxamide
MT-45	1-Myclohexyl-4-(1,2-diphenylethyl)piperazine
MXE	Methoxetamine
PV8	1-Phenyl-2-(pyrrolidin-1-yl)heptan-1-one
THF-F	Tetrahydrofuranylfentanyl
U-47700	*trans*-3,4-Dichloro-*N*-(2-(dimethylamino)cyclohexyl)-*N*-methylbenzamide
U-50488	3,4-Dichloro-*N*-methyl-*N*-[(1*R*,2*R*)-2-(1-pyrrolidinyl) cyclohexyl]-benzeneacetamide
XLR-11	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone

1 Introduction

Pharmacodynamics and pharmacokinetics of therapeutic drugs have to be studied as part of the drug-development process. Since new psychoactive substances (NPS) are not considered as therapeutic drugs (CURIA 2014), the terms pharmacodynamics and pharmacokinetics may not be appropriate. Instead, the terms toxicodynamics and toxicokinetics should be used. NPS are considered as an emerging class of compounds, mainly consumed as substitutes for old-school drugs of abuse such as amphetamine or cocaine. Hundreds of compounds have emerged on the market in the last years and the speed at which NPS appear and disappear, as well as the lack of information on their effects and harms, create substantial challenges to stakeholders interested in public health (EMCDDA 2017).

A recent and comprehensive review article discussing the toxicodynamics and toxicokinetics of NPS until mid-2016 is already available (Meyer 2016). Therefore, the present chapter aims to summarize and provide a timely update covering English-written and PubMed-listed studies published between May 2016 and November 2017 containing information about toxicokinetics of NPS. Articles mainly devoted to the elucidation of toxicodynamic properties such as the excellent work published by Simmler and Liechti who classified cathinone NPS according to their pharmacology at monoamine transporters and receptors (Simmler and Liechti 2017) will not be included as they will be part of another chapter within this Handbook of Experimental Pharmacology. The findings will be summarized starting with a view back on very recent review articles and will be followed by selected original articles.

2 Methods

A first search of PubMed for English-written literature published between 01 May 2016 and 01 November 2017 was performed using the search term ((novel psychoactive substances[Title/Abstract]) OR new psychoactive substances[Title/Abstract]) AND ("2016/05/01"[Date – Publication] : "2017/11/01"[Date – Publication]) or (STRIDA). In addition, search hits were refined further using the terms "toxicokinetic," "pharmacokinetic," "absorption," "distribution," "metabolism," and "excretion" in any field.

3 Results and Discussion

A total of 388 articles were identified by the first search. Articles mainly presenting toxicodynamic data (Gatch et al. 2017; McLaughlin et al. 2017a, b; Sahai et al. 2017) were not considered further. The second search identified six review articles and 43 original research papers published in the given time frame, which were topic-related and selected for this review. These publications are shown in Tables 1–3. In the following, first the content of the review articles will be briefly summarized with focus on the key findings. Afterwards, selected research papers are also summarized and discussed. Key points related to both can also be found in Tables 1–3.

Table 1 Selected review articles on the toxicokinetics of new psychoactive substances (NPS) published between May 2016 and November 2017

Reference	Time frame reviewed; from–to	Content in brief
Diao and Huestis (2017)	n.g.	Reviewed metabolism of synthetic cannabinoids and discussed strategies for identifying optimal urinary marker metabolites of synthetic cannabinoids intake
Ellefsen et al. (2016)	Without limiting period to 31 July 2015	Reviewed mainly metabolism but also absorption, distribution, and excretion of synthetic cathinones besides analytical methods used for detection and quantification in biological matrices, and toxicological findings from human performance and postmortem toxicology cases
Manchester et al. (2017)	n.g.	Reviewed toxicokinetics, mainly metabolism, of new psychoactive substance benzodiazepines but also discussed in detail the different chemical classes, mechanism of action, and analytical detection of therapeutically as well as new psychoactive substance benzodiazepines
Meyer (2016)	January 2015 to April 2016	Reviewed original articles and review articles covering toxicodynamics and toxicokinetics of NPS, how NPS may act in severe intoxication and their in vitro metabolism to allow detection in biological matrices
Papaseit et al. (2017)	2010 to n.g.	Reviewed mainly mephedrone concentrations in cases of fatal and nonfatal intoxications but also findings from wastewater analysis and analysis of pooled urine samples
Ventura et al. (2017)	Without limiting period to n.g.	Reviewed mainly toxicodynamics but also included some data on the metabolism and reported blood concentrations of fentanyl analogues and other opioids abused as NPS

Articles are sorted by first author's name (n.g. = not given)

3.1 Selected Review Articles on Toxicokinetics of New Psychoactive Substances Published Between May 2016 and November 2017

Besides the predecessor article (Meyer 2016), five review articles were identified and considered suitable for this section. The article by Papaseit et al. focused on one compound and summarized current toxicokinetics knowledge on mephedrone concentrations in biological samples from cases of acute intoxications, pharmacokinetic studies, wastewater, and anonymous pooled urine analysis (Papaseit et al. 2017). They included a total of 97 fatal cases and 57 nonfatal intoxication cases. Mephedrone mean blood concentrations ranged from 166 ng/mL in nonfatal cases to 2,663 ng/mL in fatal cases. Acute toxicity could be associated with mean concentrations starting at 135 ng/mL. However, great variability in the observed concentrations was most likely due to interindividual responses and particularly due to polydrug use. Ellefsen et al. reviewed

Table 2 Selected original papers on different aspects of the toxicokinetics of NPS published between May 2016 and November 2017

Reference	Compound(s) tested	Content in brief
Backberg et al. (2017b)	MDMB-CHMICA	Reported serum concentrations of MDMB-CHMICA from eight patients in the range 3.4–86.4 (median 18.6, mean 24.5 ng/mL)
Backberg et al. (2016)	3-Fluorophenmetrazine	Reported serum concentrations of 3-fluorophenmetrazine from 15 patients in the range 2.7–1,416 ng/mL
Barcelo et al. (2017)	5-MAPB 5-APB 5-EAPB Ethylphenidate Ritalinic acid	Reported hair, serum, and urine concentrations in an intoxication case Peak serum concentrations ranged from 69.3 (5-APB) to 507.7 ng/mL (ritalinic acid)
Beck et al. (2017)	Pyrovalerone derivatives	Reported serum and urine concentrations from 114 cases ranging from 1 to 300,000 ng/mL
Beck et al. (2016)	alpha-PVP	Reported concentration from 42 cases. Concentrations in serum ranged from 4.0 to 606 (median 64; $n = 42$) ng/mL and in urine from 2.0 to 41,294 (median 1,782; $n = 25$) ng/mL in urine
Coopman et al. (2016)	Ocfentanil	Reported femoral blood concentrations after death involving ocfentanil at 15.3 ng/mL
Daveluy et al. (2016)	Methiopropamine	Reported plasma concentrations 13 h after presentation to the emergency department of MPA at 14 ng/mL
Fujita et al. (2017)	alpha-PHP	Reported serial alpha-PHP serum concentrations (peak at 175 ng/mL) and elimination half-life of 37 h
Grapp et al. (2017)	MDPV	Reported serum levels from 23 cases, ranging from traces (<10 ng/mL) up to 576 ng/mL with a mean concentration of 118 ng/mL and median of 47 ng/mL Detected MDPV and its metabolites 2′-oxo-MDPV, demethylenyl-MDPV, demethylenyl-methyl-MDPV, demethylenyl-oxo-MDPV, demethylenyl-methyl-oxo-MDPV, and demethylenyl-methyl-*N*,*N*-bisdealkyl-MDPV in matched urine samples
Helander et al. (2017a)	Acrylfentanyl 4Cl-iBF 4F-iBF THF-F	Reported eight serum acrylfentanyl concentrations (0.5–2.1 ng/mL, median 0.9 ng/mL), nine urine acrylfentanyl concentrations (0.2–10.5 μg/mmol creatinine, mean 4.6 μg/mmol creatinine, median 5.2 μg/mmol creatinine). Single concentrations were reported for 4Cl-iBF, 4F-iBF, and THF-F, ranging from 5 to 45 ng/mL in serum and from 11 to 136 μg/mmol creatinine in urine
Helander et al. (2017b)	MT-45	Reported MT-45 serum concentrations from three cases at 280, 112, and 22 ng/mL

(continued)

Table 2 (continued)

Reference	Compound(s) tested	Content in brief
Hondebrink et al. (2016)	mCPP 4-FA MXE	Neurotoxicity screening
Lopez-Arnau et al. (2017)	MDPV	In vitro and in vivo interaction between MDPV and ethanol
Lucchetti et al. (2017)	cis-4,4′-DMAR	Toxicokinetic study in rats after an intravenous and intraperitoneal injection
Luethi et al. (2017)	Cathinones	Effect and cytotoxicity on two human cell lines
Olesti et al. (2017b)	Mephedrone	Controlled trial in males by oral application of 150 mg
Wagmann et al. (2017)	13 AMT analogs	Tested for potential to inhibit MAO isoforms in vitro

Papers focusing on the metabolism of NPS can be found in Table 3. Further details not listed here can be found in the text. Articles are sorted by first author's name

the toxicokinetics, analytical methods used for detection and quantification in biological matrices, and toxicological data of synthetic cathinones (Ellefsen et al. 2016). Metabolism studies were found to be mainly performed using human liver microsomes (HLM) with biotransformations being predominantly based on ketone reduction, carbonylation of the pyrrolidine ring, and further oxidative. They commented that the lack of controlled administration studies in humans complicates the interpretation of synthetic cathinone concentrations in human biofluids and that often other psychoactive substances were found in combination with synthetic cathinones, which further complicated the interpretation, which is in line with one of the conclusions drawn by Papaseit et al. (2017).

A further review discussing the metabolism of a particular NPS class was published by Diao and Huestis (2017). The paper was devoted to the metabolism of synthetic cannabinoids, which summarized metabolic patterns of different synthetic cannabinoid generations and provided a workflow for identifying specific metabolites that could serve as urinary marker for confirming their intake. This recommendation is not only valid for synthetic cannabinoids and might be generalized as follows. NPS should be incubated with human liver preparations such as hepatocytes or microsomes/cytosol and the most abundant and characteristic metabolites should be characterized by mass spectrometry. Finally, authentic positive urine specimens and/or paired blood samples should ideally be analyzed to confirm the formation of metabolite markers.

Ventura et al. reviewed the topic associated with synthetic opioids. They included fentanyl analogs, AH-7921, MT-45, U-47700, U-50488, desomorphine, mitragynine, tramadol, tapentadol, salvinorin A and its analog herkinorin (Ventura et al. 2017). They discussed their toxicodynamics, but also summarized data on their toxicokinetics such as metabolism and blood concentrations, if available.

Table 3 Selected original papers primarily studying the metabolism of NPS published between May 2016 and November 2017

Reference	Compound(s)	Content in brief
Carlier et al. (2017)	ADB-FUBINACA	Metabolites identified in: pooled human hepatocytes Major metabolic reactions: alkyl and indazole hydroxylation, terminal amide hydrolysis, glucuronide conjugations, and dehydrogenation Metabolic stability evaluated using HLM (half-life 39.7 min, hepatic clearance of 9.0 mL/min/kg, and a 0.5 extraction ratio)
Caspar et al. (2017a)	25B-NBOMe 25C-NBOMe	Metabolites identified in: rat/human urine Major metabolic reactions: *O*-demethylation, O,O-bis-demethylation, hydroxylation, glucuronidation, and sulfation
Caspar et al. (2017b)	5-MeO-2-Me-DALT 5-MeO-2-Me-ALCHT 5-MeO-2-Me-DIPT	Metabolites identified in: rat urine, HLM, HLC, recombinant human CYPs Major metabolic reactions: *O*-demethylation (CYP2C19 and 2D6), hydroxylation (CYP1A2, 2C19, 2D6, and 3A4), *N*-dealkylation (CYP2C19, 2D6, and 3A4), glucuronidation, and sulfation
Diao et al. (2017)	MN-18 5F-MN-18	Metabolites identified in: pooled human hepatocytes Most abundant metabolites: 1-pentyl-1*H*-indazole-3-carboxylic acid, pentyl-carbonylated MN-18, naphthalene-hydroxylated MN-18, 5′-OH-MN-18, MN-18 pentanoic acid, 1-(5-fluoropentyl)-1*H*-indazole-3-carboxylic acid MN-18 and 5F-MN-18 in vitro binding affinities to human CB1 and CB2 (K_i = 1.65–3.86 nmol/L)
Fabregat-Safont et al. (2017)	5-MeO-MIPT	Metabolites and pharmacokinetic identified in: mice (C57BLJ/6) *O*-demethylated and parent compound proposed as urinary markers
Franz et al. (2017)	MDMB-CHMICA	Metabolites identified in: human urine and pooled HLM Major metabolic reactions: hydrolysis of the methyl ester, oxidation of the cyclohexyl methyl side chain cAMP accumulation assay showed that it is a potent full agonist at the CB_1 receptor
Grafinger et al. (2017)	5-MeO-MiPT	Metabolites identified in: pooled HLM, blood, urine Major metabolic reactions: demethylation and hydroxylation Concentrations in blood and urine were 160 and 3,380 ng/mL, respectively
Holm et al. (2016)	omega-OH metabolite of JWH-018	JWH-018 omega-OH is a substrate for alcohol dehydrogenase (ADH) and aldehyde

(continued)

Table 3 (continued)

Reference	Compound(s)	Content in brief
		dehydrogenase (ALDH) enzymes and that oxidative defluorination is catalyzed by non-CYP enzyme(s)
Huppertz et al. (2017)	Flubromazolam	Metabolites identified in: human urine (one sample after controlled ingestions of 0.5 mg) Flubromazolam, mono-hydroxy flubromazepam, and glucuronides detected in urine
Kusano et al. (2017)	5F-ADB Diphenidine	Metabolites identified in: human blood/urine Major metabolic reactions 5F-ADB: ester hydrolysis, oxidative defluorination, oxidation to carboxylic acid; major metabolic reactions diphenidine: mono- and dihydroxylation Postmortem blood concentrations: 0.19 ± 0.04 ng/mL for 5F-ADB and 12 ± 2.6 ng/mL for diphenidine
Mardal et al. (2016)	3-Fluorophenmetrazine	Metabolites identified in: human/rat urine, recombinant human CYPs Major metabolic reactions: *N*-oxidation, aryl hydroxylation, *O*-methylation, alkyl hydroxylation, oxidation, degradation of the ethyl-bridge, glucuronidation, and sulfation Main excretion products in the human urine: unchanged compound and the *N*-oxide
Michely et al. (2017b)	DALT 5-Fluoro-DALT, 7-methyl-DALT 5,6-Methylenedioxy-DALT	Metabolites identified in: rat urine, pooled HLM, recombinant human CYP Major metabolic reactions: aromatic and aliphatic hydroxylations, *N*-dealkylation, *N*-oxidation, *O*-demethylenation, glucuronidation, sulfation Initial phase I reactions catalyzed by: CYP1A2, 2B6, 2C9, 2C19, 2D6, 3A4, 3A5
Michely et al. (2017a)	3-MeO-PCP 3-MeO-PCPy	Metabolites identified in: rat urine, pooled HLM, recombinant human CYP Major metabolic reactions: aliphatic and aromatic hydroxylations, carboxylation after ring opening, *O*-demethylation, glucuronidation Initial phase I reactions catalyzed by: CYP2B6, 2C19, CYP 2D6 (3-MeO-PCP); CYP2B6, 2C9, 2D6 (3-MeO-PCPy)
Mogler et al. (2017)	5F-MDMB-PICA	Metabolite identified in: pooled HLM, human urine Major metabolic reactions: hydroxylation, hydrolysis of terminal methyl ester Suitable urinary biomarkers: metabolites after methyl ester hydrolysis or mono-hydroxylation at the indole ring
Moosmann et al. (2016)	Adinazolam Cloniprazepam Fonazepam	Metabolites identified in: pooled HLM Most abundant metabolites: *N*-desmethyladinazolam,

(continued)

Table 3 (continued)

Reference	Compound(s)	Content in brief
	3-Hydroxyphenazepam Metizolam Nitrazolam	*N*-didesmethyladinazolam, hydroxymetizolam, dihydroxymetizolam, *N*-dealkylcloniprazepam, hydroxylcloniprazepam, aminocloniprazepam, hydroxynitrazolam, aminonitrazolam, hydroxyfonazepam, aminofonazepam, no in vitro phase I metabolites for 3-OH-phenazepam
Nielsen et al. (2017)	25I-NBOMe 25I-NBOH	Metabolites identified in: recombinant human CYPs, recombinant human monoamine oxidase, pooled HLM with and without specific CYP chemical inhibitors, single donor HLM Intrinsic clearance values: 70.1 and 118.7 mL/min/kg for 25I-NBOMe and 25I-NBOH, respectively Major metabolic reactions: *O*-demethylation, *N*-dealkylation, dehydrogenation, direct glucuronidation (25I-NBOH) Main reactions catalyzed by CYP3A4 for 25I-NBOMe and CYP2D6 for 25I-NBOH
Noble et al. (2017)	Flubromazolam	Metabolites identified in: pooled HLM and recombinant cytochrome P450 and authentic forensic samples Major metabolic reactions: hydroxylation on the alpha- and/or 4-position (CYP3A4, 3A5), glucuronidation
Olesti et al. (2017a)	Mephedrone	Metabolites identified in: human urine Most abundant metabolites: carboxy-mephedrone, nor-mephedrone, dihydro-mephedrone, 4′-carboxy-mephedrone
Pettersson Bergstrand et al. (2017)	Flubromazolam Pyrazolam	Metabolites identified in: human urine Most abundant metabolites: parent glucuronides, mono-hydroxy metabolites, mono-hydroxy glucuronides
Richter et al. (2017a)	MDMA, MBDB Butylone MDPPP MDPV MDBP 5-MAPB 5-API	Metabolites identified in: pooled HLM combined with pooled HLC or pooled human liver S9 fraction after addition of co-substrates for six phase I and II reactions
Richter et al. (2017b)	XLR-11 AB-PINACA FUB-PB-22 4-Methoxy-alpha-PVP 25I-NBOMe Meclonazepam	Metabolites identified in: pooled human S9 fraction, pooled HLM combined with pooled HLC after addition of the co-substrates for main metabolic phase I and II reactions; results compared to published data by primary human hepatocytes and human urine
Staeheli et al. (2017)	CUMYL-PINACA 5F-CUMYL-PINACA	Metabolites identified in: pooled HLM Hydroxylation major biotransformation for all

(continued)

Table 3 (continued)

Reference	Compound(s)	Content in brief
	CUMYL-4CN-BINACA 5F-CUMYL-P7AICA CUMYL-4CN-B7AICA	5 cumyl-derivatives followed by dihydroxylation Major metabolic pathway nitrile function was hydrolyzed followed by carboxylation
Steuer et al. (2017)	Butyrfentanyl	Metabolites identified in: HLM, recombinant cytochrome P450 enzymes, human blood/urine Major metabolic reactions: hydroxylation, *N*-dealkylation being the major ones in vitro CYP2D6 and 3A4 were involved in the primary metabolic reactions
Swortwood et al. (2016)	PV8	Metabolites identified in: HLM, human hepatocytes, human urine Major metabolic reactions: hydroxylation, ketone reduction, carboxylation, *N*-dealkylation, iminium formation, dehydrogenation, *N*-oxidation, and carbonylation Main metabolites in human urine: parent PV8, aliphatic hydroxylation, ketone reduction + aliphatic hydroxylation, aliphatic carboxylation
Temporal et al. (2017)	NBOMe compounds	Metabolites identified in: pooled HLM, human blood/urine Major metabolic reactions: *O*-demethylations, hydroxylations + reduction at the amine group
Wohlfarth et al. (2017)	25C-NBOMe 25I-NBOMe	Metabolites identified in: human hepatocytes, human/mouse urine Major metabolic reactions: *O*-demethylation, *O*-di-demethylation, hydroxylation, glucuronidation, sulfation

Further details not listed here can be found in the text. Articles are sorted by first author's name
HLM human liver microsomes, *HLC* human liver cytosol, *CYPs* cytochrome P450 enzymes

Finally, toxicokinetics of benzodiazepines abused as NPS were reviewed, besides their chemical data, toxicodynamic properties, and analytical data, in an article published by Manchester et al. (2017). Two very interesting sections were devoted to the property prediction by quantitative structure activity relationship modeling and to the stability of drugs during transportation and/or storage.

3.1.1 Concluding Opinion

A challenge that arises from the world of NPS is that many of them might already be outdated by the time NPS-related review articles are published (Meyer 2016). It is therefore still necessary to have frequently updated reviews available to keep pace with recent developments. It is an encouraging development that review articles dedicated to particular NPS classes are increasingly appearing, as this offers the opportunity to cover insights into the complex relationship between toxicodynamics, toxicokinetics, and analytical science because they are often depending on each other.

3.2 Selected Original Research Papers on Toxicokinetics of New Psychoactive Substances Published Between May 2016 and November 2017

The following chapter is divided into three paragraphs, which summarize case reports associated with NPS intake, different aspects of NPS' toxicokinetics, and their metabolism both based on published original papers between May 2016 and November 2017. Key aspects of selected papers can also be found as an overview in Tables 2 and 3.

3.2.1 Case Reports on New Psychoactive Substances

Interesting key information such as blood concentrations obtained from casework (Barcelo et al. 2017; Coopman et al. 2016; Daveluy et al. 2016; Fujita et al. 2017; Grapp et al. 2017) and the STRIDA project study (Backberg et al. 2016, 2017a, b; Beck et al. 2016, 2017; Helander et al. 2017a, b) can be found in Table 2. Further discussions of confirmed overdose cases and clinical aspects of NPS overdose can be found in further chapters of this handbook.

3.3 Different Aspects on Toxicokinetics of New Psychoactive Substances

A neurotoxicity screening of NPS was developed using cortical cultures grown on multi-well microelectrode arrays (Hondebrink et al. 2016). MXE, mCPP, and 4-FA decreased neuronal activity and MXE most potently inhibited neuronal activity with an IC_{50} of 0.5 μM, whereas 4-FA was least potent with an IC_{50} of 113 μM. However, directly correlating decreased neuronal activity with neurotoxicity may overestimate the toxic effects. A further study tested the effect of five frequently used cathinones on HepG2 and HepaRG cell lines (Luethi et al. 2017). Cytotoxicity and disturbed mitochondrial function, determined by adenylate kinase release and cellular adenosine-triphosphate content, respectively, were observed in both cell lines after exposure to, e.g., 4-methoxymethcathinone (methedrone) and 3,4-methylenedioxy-N-methylcathinone (methylone). Cytochrome P450 (CYP) induction (metabolism) in HepaRG cells did not increase the toxicity of the investigated compounds. Furthermore, mitochondrial toxicity by bupropion, MDPV, and naphyrone was confirmed in HepG2 cells.

Wagmann et al. tested 13 AMT analogs for their potential to inhibit MAO isoforms (Wagmann et al. 2017). All test drugs showed MAO-A inhibition (IC_{50} between 0.049 and 166 μM) and four analogs also inhibited MAO-B (IC_{50} between 82 and 376 μM). 7-Me-AMT provided the lowest IC_{50} value against MAO-A and was identified as a competitive MAO-A inhibitor.

Studies on the in vitro interaction between MDPV and ethanol using rat liver microsomes showed an enhancing effect of ethanol on MDPV metabolism increasing its metabolic rate, most likely due to interactions at microsomal level (Lopez-Arnau et al. 2017). In vivo plasma ethanol concentrations were also significantly increased by MDPV leading to a reduced ethanol elimination rate. A further study in rats was reported by Lucchetti et al., who administered 1 mg/kg body weight *cis*-4,4′-DMAR intravenously

to rats and reported plasma levels, which declined rapidly (≥80% in 4 h), followed by a slow elimination phase ($t_{1/2}$ of 5.14 h) (Lucchetti et al. 2017). After intraperitoneal injection, a t_{max} of 15 min was observed and C_{max} and $AUC_{0–240min}$ showed dose proportionality over the dose range 1–10 mg/kg body weight.

In a randomized, double-blind, cross-over controlled trial, 150 mg mephedrone was orally administered to six healthy males (Olesti et al. 2017b). Peak plasma concentrations (122.6 ± 32.9 ng/mL) were reported 0.5–2 h after drug administration and $t_{1/2}$ calculation led to value of 2.2 h.

3.4 Metabolism Studies

A total of 26 original papers were identified with a focus on the metabolism of NPS. In the following, the publications will be summarized based on the investigated NPS class.

3.4.1 Synthetic Cannabinoids

The metabolism of synthetic cannabinoids was investigated in eight studies (Carlier et al. 2017; Diao et al. 2017; Franz et al. 2017; Holm et al. 2016; Kusano et al. 2017; Mogler et al. 2017; Richter et al. 2017b; Staeheli et al. 2017). Pooled human hepatocytes were used two times, pooled HLM five times, pooled human liver cytosol (HLC) two times, pooled human S9 fraction once, and authentic human urine was available three times. Studies were mainly aimed at identifying metabolites for toxicological screening procedures in human urine samples. Richter et al. compared different tools for this purpose and concluded that "Human liver preparations and particularly the pooled S9 fraction could be shown to be a sufficient . . . alternative in context of metabolism studies also for developing toxicological urine screenings." (Richter et al. 2017b). Five cumyl-based synthetic cannabinoid derivatives were investigated for an in vitro phase I metabolism study with a focus on the analytical differentiation and interpretation of the metabolites (Staeheli et al. 2017). The authors concluded that some of the expected biotransformations led to structurally identical metabolites, which should therefore not be used as marker for the intake of a specific parent compound. In general, synthetic cannabinoids were again found to be extensively metabolized and that analytically used urinary biomarkers need to be metabolites rather than parent compounds.

In contrast to most of the studies, Holm et al. were not interested in finding suitable biomarkers (Holm et al. 2016). They investigated the metabolic origin of the omega-carboxylic acid metabolite of JWH-018, which is considered a major human urinary metabolite. As this metabolite is not formed to any high extent in HLM incubations, the authors assumed the involvement of cytosolic enzymes. Using incubations with HLC, it was shown that the omega-hydroxy metabolite of JWH-018, and in contrast to the parent compound, was metabolized by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) and that the omega-carboxylic acid metabolite of JWH-018 was formed in HLC. Further trapping tests proved the presence of the

aldehyde intermediate. They also assumed that the oxidative defluorination of AM-2201 was catalyzed by non-CYP enzyme(s).

3.4.2 NBOMe Analogs, Tryptamines, and Phencyclidines

A total of ten original papers reported on the metabolism of hallucinogenic NBOMes ($n = 5$), tryptamines ($n = 4$), and dissociative phencyclidines ($n = 1$) (Caspar et al. 2017a, b; Fabregat-Safont et al. 2017; Grafinger et al. 2017; Michely et al. 2017a; Michely et al. 2017b; Nielsen et al. 2017; Richter et al. 2017b; Temporal et al. 2017; Wohlfarth et al. 2017). Again, HLM ($n = 8$) were the most frequently used in vitro tool. Several studies included in vivo data obtained from rat urine ($n = 4$) and/or human urine ($n = 3$). The NBOMe derivatives were, similar to the synthetic cannabinoids, found to be extensively metabolized and analytical methods therefore need to include metabolites. Their extensive metabolism is underlined by the fact that Caspar et al. were able to identify 66 metabolites for 25B-NBOMe and 69 for 25C-NBOMe (Caspar et al. 2017a). Similarly, tryptamine derivatives underwent an extensive metabolism as exemplified again in studies by Caspar et al. who identified 24 phase I and 12 phase II metabolites for 5-MeO-2-Me-DALT, 24 phase I and 14 phase II metabolites for 5-MeO-2-Me-ALCHT, and 20 phase I and 11 phase II metabolites for 5-MeO-2-Me-DIPT (Caspar et al. 2017a). Main reactions were *O*-demethylation, hydroxylation, *N*-dealkylation, and combinations thereof as well as glucuronidation and sulfation of phase I metabolites. Several studies also included the identification of CYP isoenzymes and not surprisingly, CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4 were involved in at least one initial step in the metabolism of NBOMes, tryptamines ($n = 4$), and/or phencyclidines (Caspar et al. 2017b; Michely et al. 2017a, b; Nielsen et al. 2017). In a study linked to an intoxication case with 5-MeO-MIPT, Grafinger et al. included both in vitro and in vivo metabolism data, in addition to 5-MeO-MiPT concentrations detected in blood and urine, which were 160 and 3,380 ng/mL, respectively (Grafinger et al. 2017).

3.4.3 New Psychoactive Substances Benzodiazepines, Psychostimulants, and Miscellaneous

The metabolism of NPS benzodiazepines was studied for flubromazolam (Huppertz et al. 2017; Noble et al. 2017; Pettersson Bergstrand et al. 2017), adinazolam, cloniprazepam, fonazepam, 3-hydroxyphenazepam, metizolam and nitrazolam (Moosmann et al. 2016), pyrazolam (Pettersson Bergstrand et al. 2017), and meclonazepam (Richter et al. 2017b). Further metabolism data were published for the stimulants 3-fluorophenmetrazine (Mardal et al. 2016), mephedrone (Olesti et al. 2017a), butylone, MDPPP, MDPV, MDBP, 5-MAPB, 5-API (Richter et al. 2017a), 4-methoxy-alpha-PVP (Richter et al. 2017b), and PV8 (Swortwood et al. 2016) as well as for diphenidine (Kusano et al. 2017) and the fentanyl derivative butyrfentanyl (Steuer et al. 2017).

The metabolism study by Huppertz et al. also included some toxicokinetic data after single oral ingestion of 0.5 mg flubromazolam (Huppertz et al. 2017). Flubromazolam and its mono-hydroxylated metabolite were detectable in urine for up to 6.5 and 8 days, respectively. The terminal elimination half-life could be estimated to fall in the range of 10–20 h and serum concentrations 8 h post-ingestion were 8 ng/mL. Flubromazolam

was also detectable in hair samples collected 2 weeks after drug uptake. Noble et al. added further toxicokinetic data of flubromazolam (Noble et al. 2017). They determined the unbound fraction of flubromazolam in pooled HLM and in plasma with subsequent prediction of its hepatic clearance (CLH). The predicted CLH of flubromazolam using well-stirred and parallel-tube models were 0.42 and 0.43 mL/min/kg, respectively. Flubromazolam is expected to be mainly metabolized by CYP3A4/5 in vivo with a high protein-binding and a low clearance.

In vivo mephedrone metabolites were reported after ingestion of 150 mg mephedrone by six males within a clinical trial (Olesti et al. 2017a). Carboxy-mephedrone and normephedrone were the most abundant metabolites found in plasma. In urine, 4′-carboxymephedrone was the main excretion product, which is in line with previous case reports.

Apart from qualitative metabolism studies using HLM, initial screening experiments with recombinant CYPs were carried out for butyrylfentanyl (Steuer et al. 2017). This drug was shown to undergo several metabolic steps such as hydroxylation and *N*-dealkylation in vitro and hydroxylation of the butanamide side chain followed by subsequent oxidation to the carboxylic acid in vivo. The initial screening experiments indicated that mainly CYP2D6 and 3A4 were involved in the primary metabolic steps. Diphenidine postmortem blood concentrations measured using standard addition were reported by Kusano et al. at 12 ± 2.6 ng/mL (Kusano et al. 2017).

3.4.4 Concluding Opinion

Performing qualitative in vitro metabolism studies with NPS still provide important data, are well-documented, and are comparably easy to carry out. However, the choice of the used metabolizing system is crucial. It is obviously not of great relevance whether S9, HLM/HLC, or hepatocytes are used but the importance lies in adding all important co-actors to the artificial systems (HLM/HLC/S9), which then allow formation of the most important metabolites (Richter et al. 2017a, b).

Furthermore, controlled in vivo studies on the toxicokinetics of NPS in humans are still not available although some encouraging exceptions exist (Olesti et al. 2017a, b). Therefore, the implementation of in vitro studies has to be used as a substitute but one has to be mindful that data interpretation can be complicated as, for example, the amount of NPS ingested by users is often not known and can be highly variable from user to user, not to mention that co-consumption of NPS with other compounds frequently occurs, thus, impacting interpretation (Ellefsen et al. 2016; Meyer 2016; Papaseit et al. 2017). In vivo animal studies have similar limitations, in addition to interspecies differences. Nevertheless, metabolism studies are of outstanding importance to allow for reliable detection of NPS and/or their metabolites in biological matrices, particularly in urine.

In the case of human data derived from single case reports, it is also very important to consider important limitations. Such data may be derived from severe overdosing, individuals may have had single polymorphisms, or samples may have originated from partly decomposed postmortem material. All of these parameters might limit the ability to generalize some of these data.

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References

Backberg M, Westerbergh J, Beck O, Helander A (2016) Adverse events related to the new psychoactive substance 3-fluorophenmetrazine – results from the Swedish STRIDA project. Clin Toxicol (Phila) 54:819–825

Backberg M, Jonsson KH, Beck O, Helander A (2017a) Investigation of drug products received for analysis in the Swedish STRIDA project on new psychoactive substances. Drug Test Anal. https://doi.org/10.1002/dta.2226

Backberg M, Tworek L, Beck O, Helander A (2017b) Analytically confirmed intoxications involving MDMB-CHMICA from the STRIDA project. J Med Toxicol 13:52–60

Barcelo B, Gomila I, Rotolo MC et al (2017) Intoxication caused by new psychostimulants: analytical methods to disclose acute and chronic use of benzofurans and ethylphenidate. Int J Legal Med 131:1543–1553

Beck O, Franzen L, Backberg M, Signell P, Helander A (2016) Toxicity evaluation of alpha-pyrrolidinovalerophenone (alpha-PVP): results from intoxication cases within the STRIDA project. Clin Toxicol (Phila) 54:568–575

Beck O, Backberg M, Signell P, Helander A (2017) Intoxications in the STRIDA project involving a panorama of psychostimulant pyrovalerone derivatives, MDPV copycats. Clin Toxicol (Phila). https://doi.org/10.1080/15563650.2017.1370097

Carlier J, Diao X, Wohlfarth A, Scheidweiler K, Huestis MA (2017) In vitro metabolite profiling of ADB-FUBINACA, a new synthetic cannabinoid. Curr Neuropharmacol 15:682–691

Caspar AT, Brandt SD, Stoever AE, Meyer MR, Maurer HH (2017a) Metabolic fate and detectability of the new psychoactive substances 2-(4-bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl] ethanamine (25B-NBOMe) and 2-(4-chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl] ethanamine (25C-NBOMe) in human and rat urine by GC-MS, LC-MS(n), and LC-HR-MS/MS approaches. J Pharm Biomed Anal 134:158–169

Caspar AT, Gaab JB, Michely JA, Brandt SD, Meyer MR, Maurer HH (2017b) Metabolism of the tryptamine-derived new psychoactive substances 5-MeO-2-Me-DALT, 5-MeO-2-Me-ALCHT, and 5-MeO-2-Me-DIPT and their detectability in urine studied by GC-MS, LC-MS(n), and LC-HR-MS/MS. Drug Test Anal. https://doi.org/10.1002/dta.2197

Coopman V, Cordonnier J, De Leeuw M, Cirimele V (2016) Ocfentanil overdose fatality in the recreational drug scene. Forensic Sci Int 266:469–473

CURIA (2014) Court of Justice of the European Union (C-358/13 and C-181/14). vol 2014/07/10

Daveluy A, Castaing N, Cherifi H et al (2016) Acute methiopropamine intoxication after "synthacaine" consumption. J Anal Toxicol 40:758–760

Diao X, Huestis MA (2017) Approaches, challenges, and advances in metabolism of new synthetic cannabinoids and identification of optimal urinary marker metabolites. Clin Pharmacol Ther 101:239–253

Diao X, Carlier J, Zhu M, Huestis MA (2017) Human hepatocyte metabolism of novel synthetic cannabinoids MN-18 and its 5-fluoro analog 5F-MN-18. Clin Chem 63:1753–1763

Ellefsen KN, Concheiro M, Huestis MA (2016) Synthetic cathinone pharmacokinetics, analytical methods, and toxicological findings from human performance and postmortem cases. Drug Metab Rev 48:237–265

EMCDDA (2017) European drug report 2017: trends and developments publications of the European Union. European Monitoring Centre for Drugs and Drug Addiction, Luxembourg

Fabregat-Safont D, Barneo-Munoz M, Martinez-Garcia F, Sancho JV, Hernandez F, Ibanez M (2017) Proposal of 5-methoxy-N-methyl-N-isopropyltryptamine consumption biomarkers through identification of in vivo metabolites from mice. J Chromatogr A 1508:95–105

Franz F, Angerer V, Moosmann B, Auwarter V (2017) Phase I metabolism of the highly potent synthetic cannabinoid MDMB-CHMICA and detection in human urine samples. Drug Test Anal 9:744–753

Fujita Y, Mita T, Usui K et al (2017) Toxicokinetics of the synthetic cathinone alpha-pyrrolidinohexanophenone. J Anal Toxicol. https://doi.org/10.1093/jat/bkx080

Gatch MB, Dolan SB, Forster MJ (2017) Locomotor activity and discriminative stimulus effects of a novel series of synthetic cathinone analogs in mice and rats. Psychopharmacology (Berl) 234:1237–1245

Grafinger KE, Hadener M, Konig S, Weinmann W (2017) Study of the in vitro and in vivo metabolism of the tryptamine 5-MeO-MiPT using human liver microsomes and real case samples. Drug Test Anal. https://doi.org/10.1002/dta.2245

Grapp M, Kaufmann C, Ebbecke M (2017) Toxicological investigation of forensic cases related to the designer drug 3,4-methylenedioxypyrovalerone (MDPV): detection, quantification and studies on human metabolism by GC-MS. Forensic Sci Int 273:1–9

Helander A, Backberg M, Signell P, Beck O (2017a) Intoxications involving acrylfentanyl and other novel designer fentanyls – results from the Swedish STRIDA project. Clin Toxicol (Phila) 55:589–599

Helander A, Bradley M, Hasselblad A et al (2017b) Acute skin and hair symptoms followed by severe, delayed eye complications in subjects using the synthetic opioid MT-45. Br J Dermatol 176:1021–1027

Holm NB, Noble C, Linnet K (2016) JWH-018 omega-OH, a shared hydroxy metabolite of the two synthetic cannabinoids JWH-018 and AM-2201, undergoes oxidation by alcohol dehydrogenase and aldehyde dehydrogenase enzymes in vitro forming the carboxylic acid metabolite. Toxicol Lett 259:35–43

Hondebrink L, Verboven AHA, Drega WS et al (2016) Neurotoxicity screening of (illicit) drugs using novel methods for analysis of microelectrode array (MEA) recordings. Neurotoxicology 55:1–9

Huppertz LM, Moosmann B, Auwarter V (2017) Flubromazolam – basic pharmacokinetic evaluation of a highly potent designer benzodiazepine. Drug Test Anal. https://doi.org/10.1002/dta.2203

Kusano M, Zaitsu K, Taki K et al (2017) Fatal intoxication by 5F-ADB and diphenidine: detection, quantification, and investigation of their main metabolic pathways in humans by LC/MS/MS and LC/Q-TOFMS. Drug Test Anal. https://doi.org/10.1002/dta.2215

Lopez-Arnau R, Buenrostro-Jauregui M, Munoz-Villegas P et al (2017) The combination of MDPV and ethanol results in decreased cathinone and increased alcohol levels. Study of such pharmacological interaction. Prog Neuropsychopharmacol Biol Psychiatry 76:19–28

Lucchetti J, Marzo CM, Di Clemente A, Cervo L, Gobbi M (2017) A validated, sensitive HPLC-MS/MS method for quantification of cis-para-methyl-4-methylaminorex (cis-4,4′-DMAR) in rat and human plasma: application to pharmacokinetic studies in rats. Drug Test Anal 9:870–879

Luethi D, Liechti ME, Krahenbuhl S (2017) Mechanisms of hepatocellular toxicity associated with new psychoactive synthetic cathinones. Toxicology 387:57–66

Manchester KR, Lomas EC, Waters L, Dempsey FC, Maskell PD (2017) The emergence of new psychoactive substance (NPS) benzodiazepines: a review. Drug Test Anal. https://doi.org/10.1002/dta.2211

Mardal M, Miserez B, Bade R et al (2016) 3-Fluorophenmetrazine, a fluorinated analogue of phenmetrazine: studies on in vivo metabolism in rat and human, in vitro metabolism in human CYP isoenzymes and microbial biotransformation in Pseudomonas Putida and wastewater using GC and LC coupled to (HR)-MS techniques. J Pharm Biomed Anal 128:485–495

McLaughlin G, Morris N, Kavanagh PV et al (2017a) Analytical characterization and pharmacological evaluation of the new psychoactive substance 4-fluoromethylphenidate (4F-MPH) and differentiation between the (+/−)-threo and (+/−)-erythro diastereomers. Drug Test Anal 9:347–357

McLaughlin G, Morris N, Kavanagh PV et al (2017b) Synthesis, characterization and monoamine transporter activity of the new psychoactive substance mexedrone and its N-methoxy positional isomer, N-methoxymephedrone. Drug Test Anal 9:358–368

Meyer MR (2016) New psychoactive substances: an overview on recent publications on their toxicodynamics and toxicokinetics. Arch Toxicol 90:2421–2444

Michely JA, Brandt SD, Meyer MR, Maurer HH (2017a) Biotransformation and detectability of the new psychoactive substances N,N-diallyltryptamine (DALT) derivatives 5-fluoro-DALT, 7-methyl-

DALT, and 5,6-methylenedioxy-DALT in urine using GC-MS, LC-MS(n), and LC-HR-MS/MS. Anal Bioanal Chem 409:1681–1695

Michely JA, Manier SK, Caspar AT, Brandt SD, Wallach J, Maurer HH (2017b) New psychoactive substances 3-methoxyphencyclidine (3-MeO-PCP) and 3-methoxyrolicyclidine (3-MeO-PCPy): metabolic fate elucidated with rat urine and human liver preparations and their detectability in urine by GC-MS, "LC-(high resolution)-MSn" and "LC-(high resolution)-MS/MS". Curr Neuropharmacol 15:692–712

Mogler L, Franz F, Rentsch D et al (2017) Detection of the recently emerged synthetic cannabinoid 5F-MDMB-PICA in 'legal high' products and human urine samples. Drug Test Anal. https://doi.org/10.1002/dta.2201

Moosmann B, Bisel P, Franz F, Huppertz LM, Auwarter V (2016) Characterization and in vitro phase I microsomal metabolism of designer benzodiazepines – an update comprising adinazolam, cloniprazepam, fonazepam, 3-hydroxyphenazepam, metizolam and nitrazolam. J Mass Spectrom 51:1080–1089

Nielsen LM, Holm NB, Leth-Petersen S, Kristensen JL, Olsen L, Linnet K (2017) Characterization of the hepatic cytochrome P450 enzymes involved in the metabolism of 25I-NBOMe and 25I-NBOH. Drug Test Anal 9:671–679

Noble C, Mardal M, Bjerre Holm N, Stybe Johansen S, Linnet K (2017) In vitro studies on flubromazolam metabolism and detection of its metabolites in authentic forensic samples. Drug Test Anal 9:1182–1191

Olesti E, Farre M, Papaseit E et al (2017a) Pharmacokinetics of mephedrone and its metabolites in human by LC-MS/MS. AAPS J 19:1767–1778

Olesti E, Pujadas M, Papaseit E et al (2017b) GC-MS quantification method for mephedrone in plasma and urine: application to human pharmacokinetics. J Anal Toxicol 41:100–106

Papaseit E, Olesti E, de la Torre R, Torrens M, Farre M (2017) Mephedrone concentrations in cases of clinical intoxication. Curr Pharm Des. https://doi.org/10.2174/1381612823666170704130213

Pettersson Bergstrand M, Meyer MR, Beck O, Helander A (2017) Human urinary metabolic patterns of the designer benzodiazepines flubromazolam and pyrazolam studied by liquid chromatography-high resolution mass spectrometry. Drug Test Anal. https://doi.org/10.1002/dta.2243

Richter LHJ, Flockerzi V, Maurer HH, Meyer MR (2017a) Pooled human liver preparations, HepaRG, or HepG2 cell lines for metabolism studies of new psychoactive substances? A study using MDMA, MDBD, butylone, MDPPP, MDPV, MDPB, 5-MAPB, and 5-API as examples. J Pharm Biomed Anal 143:32–42

Richter LHJ, Maurer HH, Meyer MR (2017b) New psychoactive substances: studies on the metabolism of XLR-11, AB-PINACA, FUB-PB-22, 4-methoxy-alpha-PVP, 25-I-NBOMe, and meclonazepam using human liver preparations in comparison to primary human hepatocytes, and human urine. Toxicol Lett 280:142–150

Sahai MA, Davidson C, Khelashvili G et al (2017) Combined in vitro and in silico approaches to the assessment of stimulant properties of novel psychoactive substances – the case of the benzofuran 5-MAPB. Prog Neuropsychopharmacol Biol Psychiatry 75:1–9

Simmler LD, Liechti ME (2017) Interactions of Cathinone NPS with human transporters and receptors in transfected cells. Curr Top Behav Neurosci 32:49–72

Staeheli SN, Poetzsch M, Veloso VP et al (2017) In vitro metabolism of the synthetic cannabinoids CUMYL-PINACA, 5F-CUMYL-PINACA, CUMYL-4CN-BINACA, 5F-CUMYL-P7AICA and CUMYL-4CN-B7AICA. Drug Test Anal. https://doi.org/10.1002/dta.2298

Steuer AE, Williner E, Staeheli SN, Kraemer T (2017) Studies on the metabolism of the fentanyl-derived designer drug butyrfentanyl in human in vitro liver preparations and authentic human samples using liquid chromatography-high resolution mass spectrometry (LC-HRMS). Drug Test Anal 9:1085–1092

Swortwood MJ, Ellefsen KN, Wohlfarth A et al (2016) First metabolic profile of PV8, a novel synthetic cathinone, in human hepatocytes and urine by high-resolution mass spectrometry. Anal Bioanal Chem 408:4845–4856

Temporal KH, Scott KS, Mohr ALA, Logan BK (2017) Metabolic profile determination of NBOMe compounds using human liver microsomes and comparison with findings in authentic human blood and urine. J Anal Toxicol 41:646–657

Ventura L, Carvalho F, Dinis-Oliveira RJ (2017) Opioids in the frame of new psychoactive substances network: a complex pharmacological and toxicological issue. Curr Mol Pharmacol. https://doi.org/10.2174/1874467210666170704110146

Wagmann L, Brandt SD, Kavanagh PV, Maurer HH, Meyer MR (2017) In vitro monoamine oxidase inhibition potential of alpha-methyltryptamine analog new psychoactive substances for assessing possible toxic risks. Toxicol Lett 272:84–93

Wohlfarth A, Roman M, Andersson M et al (2017) 25C-NBOMe and 25I-NBOMe metabolite studies in human hepatocytes, in vivo mouse and human urine with high-resolution mass spectrometry. Drug Test Anal 9:680–698

Epidemiology of NPS Based Confirmed Overdose Cases: The STRIDA Project

Anders Helander and Matilda Bäckberg

Contents

Abstract

The Swedish STRIDA project on new psychoactive substances (NPS) monitored the occurrence and health hazards of novel recreational drugs in Sweden through evaluation of analytically confirmed adverse events presenting in emergency departments and intensive care units. During a ~6-year period from 2010 to early 2016, about 2,600 cases of suspected NPS intoxications were included in the project. About 75% of patients were men and the total age range was 8–71 (median 24) years and 57% were 25 years or younger. A large number of NPS belonging to many different drug classes were identified in project samples of

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urine and blood (serum/plasma) submitted for free drug testing, including synthetic cannabinoid receptor agonists, stimulants, cathinones, hallucinogens, dissociative drugs, benzodiazepines, and opioids, and also in drug materials from the cases forwarded to the laboratory along with the biological samples. The STRIDA project has been shown to serve as an effective early warning system for NPS by collecting data on incidence, distribution, and adverse effects and has supported healthcare professionals in the knowledge and critical care of intoxications caused by a wide range of novel substances. The results of the STRIDA project have also illustrated how drug regulations can drive the NPS market.

Keywords
Designer drugs · Internet drugs · Mass spectrometry methods · New psychoactive substances · NPS

1 Background

In the end of 2008, herbal smoking mixtures sold under the brand name "Spice" producing cannabis-like effects were demonstrated to be laced with synthetic cannabinoid receptor agonists (SCRAs) originally developed as therapeutic drug candidates (Auwärter et al. 2009; EMCDDA 2009; Marriott and Huffman 2008). The SCRA additives involved soon became controlled in some countries but were then rapidly replaced by other, yet uncontrolled structural variants to maintain the legal status of products ("legal highs") (Lindigkeit et al. 2009). This turned out to be the starting point for a new and ongoing recreational drug era, where novel "designed" recreational drugs, collectively referred to as "new psychoactive substances" or NPS, were introduced and sold openly on the surface web ("Internet drugs") to replace those that became controlled. Marketing NPS as masked products (e.g., bath salt, plant food, or research chemical), use of product labels such as "not for human consumption" and "for laboratory reference only," and production in uncontrolled contract laboratories mean that they escape the strict safety and quality control requirements commonly in place for medicinal products.

Information on literally thousands of uncontrolled substances with potential of being psychoactive and abused (e.g., neurotransmitter receptor agonists and reuptake inhibitors) is available through the scientific literature and in patent applications. From 2009 until 2016, a total of more than 530 unique NPS have been notified for the first time by the European Union early warning system (EWS) operated by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and Europol, and more than 600 substances are currently being monitored (EMCDDA 2017). From a level of typically less than 15 new substances detected per year, the number increased to 24 in 2009 and continued upwards to peak at 101 in 2014 and 98 in 2015, after which the number decreased to 66 in 2016.

Besides the large number of different substances introduced, also has the type of chemical classes widened from initially mainly SCRAs (often referred to as "spice") and synthetic stimulants (e.g., substituted cathinones; also referred to as "bath salts"

particularly in the USA) to include other commonly abused drugs such as benzodiazepines (Meyer et al. 2016; Pettersson Bergstrand et al. 2017a), hallucinogenic and dissociative drugs (Bäckberg et al. 2015a; Helander et al. 2015), and opioids (Bäckberg et al. 2015b; Helander et al. 2014a, 2016).

The NPS phenomenon represents an alarming and growing health concern in many countries. For most new substances introduced, there is no or only limited pharmacological and toxicological information available, increasing the risk for harmful adverse events and drug-related deaths to occur. Particularly for branded products, information about the content is often not listed on the product label nor analytically confirmed (Baron et al. 2011; Bäckberg et al. 2015b, 2018; Davies et al. 2010), and some NPS are sold as counterfeit or fake medicines (Drug Enforcement Administration 2016), thus adding to the risk for adverse events. Indeed, dealing with NPS-associated medical complications has become an increasing problem at emergency departments (ED) and intensive care units (ICU), although the real cause may often be unknown since routine drug testing of NPS is rarely performed (Helander et al. 2013, 2014b; Kamijo et al. 2014).

The increasing incidence of NPS opioids appearing in recent years is especially alarming, as substances of this drug class (e.g., heroin, methadone, oxycodone, tramadol, and fentanyl) are responsible for a large proportion of drug-related morbidity and mortality (Gladden et al. 2016; Ojanperä et al. 2016; Simonsen et al. 2015). The danger inherent to opioids is their high potency (potent μ-opioid receptor agonism) and rapid onset of action, and that the symptoms associated with overdose (i.e., decreased consciousness and respiratory depression) may be potentially life-threatening unless the patient quickly receives medical attention (e.g., naloxone administration) and/or hospital care (UNODC 2017; WHO 2014).

In Sweden, a nationwide project called STRIDA was started in 2010, aiming to monitor the increasing occurrence and potential health hazards associated with NPS use in the country, through evaluation of analytically confirmed serious adverse events presenting in ED and ICU (Helander et al. 2013, 2014b). This work presents a summary of results from 2010 until the first quarter of 2016, when the scope of the project unfortunately decreased considerably due to lack of funding.

2 Methods

2.1 Selection of Cases and Data Acquisition

STRIDA (the name is an acronym of the Swedish project name, but the word also means "to fight") is a cooperative project run by the Karolinska Institutet, the Karolinska University Laboratory and the Swedish Poisons Information Centre (PIC) in Stockholm. The PIC is a 24/7 nationwide toxicological telephone consultation service open for health care professionals and the public. When the PIC is called, specially trained pharmacists and medical doctors (commonly an anesthesiologist) are consulted.

Before the start of the STRIDA project in January 2010, all Swedish ED and ICU departments were sent an informative letter detailing its purpose and extent, and they received a special laboratory request form allowing free-of-charge analysis of psychoactive substances. Information about the project was also given whenever the PIC was consulted with inquiries regarding drug-related overdose cases. Patients who voluntarily admitted intake of NPS or related products or for whom intoxication with such drugs was suspected by the medical staff were invited to participate in the project. Participation required a contact by the hospital with the PIC to obtain a unique case code number to ensure patient anonymity, and biological samples (urine and/or serum/plasma) had to be submitted for analysis.

During consultation, the PIC staff recorded case notes on the age, gender, symptoms, and treatment. Whenever available, patient self-reports on the substance and/or branded name, the dose, time of intake, and route of administration were also collected. The medical staff were encouraged to collect samples of urine and serum/plasma according to standard routines for drug testing as soon as possible after admission to the ED/ICU and forward them along with the laboratory request form to the Department of Clinical Pharmacology, Karolinska University Laboratory Huddinge, Stockholm. On arrival in the laboratory, aliquots of the urine sample were immediately taken for analysis of psychoactive substance contents, as detailed below, whereas the remaining sample volume and the serum/plasma samples were stored frozen until further used.

Additional clinical and treatment information were retrieved from the PIC consultation records and requested from hospital medical records. The severity of intoxication was graded retrospectively using the Poisoning Severity Score (PSS) (Persson et al. 1998), and the level of consciousness using the Reaction Level Scale (RLS) or the Glasgow Coma Scale (GCS) (Starmark et al. 1988).

The STRIDA patients were anonymized and linked to the individual case by a code number. The project was conducted in accordance with the Helsinki Declaration and approved by the regional ethical review board (Nr. 2013/116–31/2).

2.2 Collection of Drug Materials

Drug materials brought to hospital or handed over to the ambulance personnel by the patient or accompanying persons were sometimes forwarded to the laboratory (with permission) along with the biological samples. The PIC did not routinely ask for the presence of drug materials, but this possibility was indicated on the STRIDA laboratory request form. In the laboratory, the materials were visually inspected and classified by formulation type (e.g., powder, crystal, tablet, capsule, blotter, herbal material, liquid, or other), and stored frozen until taken for analysis.

Additionally, drug products with a claimed content of novel, uncontrolled NPS were sometimes purchased from online vendors to be utilized as qualitative reference material until a certified material became available. Following analytical confirmation of the content (see below), the materials were stored frozen.

2.3 Laboratory Analysis

The biological samples received in the STRIDA project were subjected to a comprehensive toxicological analysis at the Karolinska University Laboratory, Department of Clinical Pharmacology in Huddinge, Stockholm. The analysis involved a combination of routine immunochemical assays targeting classical drugs of abuse, multicomponent liquid chromatographic–tandem mass spectrometric (LC–MS/MS), and LC–high-resolution single and tandem MS (LC–HRMS(/MS)) screening and confirmation methods targeting NPS, classical drugs, plant- and mushroom-derived substances, and prescription pharmaceuticals, as detailed elsewhere (Al-Saffar et al. 2013; Björnstad et al. 2009; Helander et al. 2013; Stephanson et al. 2017). The exact number of covered substances varied over time, as the LC–HRMS methods were continuously updated when new drugs were introduced and reference materials were available. To demonstrate the occurrence of newly identified substances, retrospective evaluation of HRMS data, or reanalysis of samples, was necessary. The lower quantification limit of the MS methods ranged from <0.5 ng/mL (e.g., for fentanyls) upwards.

Analysis of NPS materials brought to hospital by the STRIDA patients and forwarded to the laboratory together with the biological samples and of purchased products was carried out using LC–HRMS/MS at the Karolinska University Laboratory, and by LC–quadrupole-time-of-flight–MS/MS and nuclear magnetic resonance (NMR) spectroscopy at the Swedish Medical Products Agency in Uppsala (Bäckberg et al. 2018; Johansson et al. 2014).

The laboratory investigation of drug materials provided information on substance identity, but not on pharmacologically inactive components (e.g., binders in tablets and diluents or fillers in liquids, powders, and capsules), bacteria, viruses, minerals, or metals. Quantitative analysis was only performed for a few products intended for use as preliminary reference materials.

3 Results and Discussion

This work comprises STRIDA data from acute intoxication cases self-reported or suspected to be associated with NPS presenting in ED and ICU in Sweden from January 2010 until the first quarter of 2016.

3.1 Poisons Information Centre Consultations on New Psychoactive Substances

When the STRIDA project was started, the PIC organized an internal working group specially focusing on the novel recreational drugs, as the number of consultations related to NPS or unknown drugs had shown a marked increase since 2007, while remaining constant for the classical illicit drugs of abuse (Lindeman et al. 2014). A general lack of knowledge among clinicians about the many different novel drugs

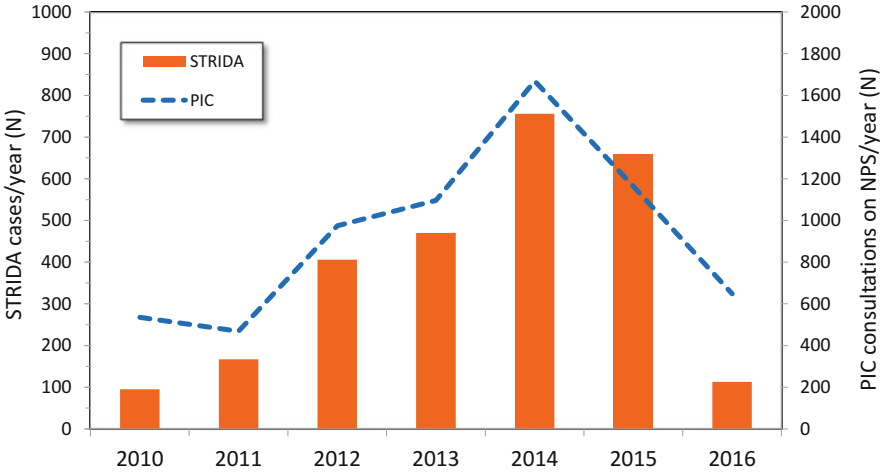

Fig. 1 Statistics of yearly numbers of acute intoxication cases analyzed in the Swedish STRIDA project on new psychoactive substances (NPS) in 2010–2016 (*bars*, *left axis*), and numbers of telephone consultations on intoxications by NPS or unknown drugs to the Swedish Poisons Information Centre (PIC) (*broken line*, *right axis*). The STRIDA project was put on a halt in early 2016 due to lack of funding, explaining the considerable drop in cases this year

appearing obviously increased the demands for PIC consultation. Once a new substance was noticed at the PIC from inquiries or shared EWS information, the internal documentation and treatment guidelines were updated and brief reports on case progress and recovery were generated and the laboratory was informed.

During the study period, the yearly number of consultations related to NPS or unknown substances increased from a baseline level of ~550 to peak at ~1,700 in 2014 (Fig. 1), indicating that in 2014, on average at least 4–5 patients needed acute hospital care in Sweden due to a suspected NPS intoxication per day. The interests in taking part in the STRIDA project rapidly spread across hospitals and, eventually, the PIC was sometimes contacted only to retrieve a project code number and admittance of free-of-charge drug testing without the need of specific treatment advice. This indicated an interest among hospital care givers to contribute to the knowledge and treatment of NPS toxicity, as the drug testing results typically were not available until after discharge of patients from the ED/ICU. However, when the possibility for free-of-charge drug testing ended in 2016 due to lack of funding, the number of PIC consultations on NPS soon resumed to the starting level.

3.2 STRIDA Project Cases

During the ~6-year study period, more than 2,600 patients presenting at Swedish ED/ICU with a suspected intoxication by NPS or unknown drugs of abuse were included in the STRIDA project, meaning that biological samples were sent to the

laboratory for toxicological investigation. The number of drug intoxication cases enrolled in the STRIDA project increased by year from 95 in 2010 to peak at 750 in 2014 (Fig. 1). Accordingly, each year, only about half or less of all PIC consultations related to a suspected intoxication by NPS were analytically confirmed in the STRIDA project.

Overall, about 75% of the STRIDA patients were men, and the total age range was 8–71 years with 57% aged 25 years or younger. The mean (median) age for male patients was 27.9 (25.0) years compared with 24.5 (21.5) years for the females. The STRIDA cases originated from all over Sweden, roughly in proportion to the regional distribution of inhabitants in the country.

A large number of NPS belonging to many different drug classes were identified in the samples of urine and/or blood submitted for free drug testing including SCRAs (Bäckberg et al. 2017), stimulants and cathinones (Beck et al. 2015, 2016; Bäckberg et al. 2014, 2015c, 2016), hallucinogens and dissociative drugs (Bäckberg et al. 2015a), benzodiazepines (Pettersson Bergstrand et al. 2017a, b), and opioids (Bäckberg et al. 2015b; Helander et al. 2014a, 2016, 2017b) as well as classical illicit drugs and ethanol. Mixing of several drugs at the same time was very common.

3.3 Time Course of New Psychoactive Substances in the STRIDA Project

Since the start of the STRIDA project, the set of psychoactive substances detected has varied over time, mainly in response to substance classification which in Sweden is done individually (Helander et al. 2013; Helander and Bäckberg 2016), and typically only a few times per year. When control measures of NPS begin to apply, substances are removed from the market and replaced with other structural variants, which complicates the analytical work (Bäckberg et al. 2015a, c; Helander and Bäckberg 2016).

Each year, the PIC consultations related to NPS (i.e., potential STRIDA cases) mainly concerned SCRAs ("spice"), cathinones, or "unknown drugs," although there were fluctuations over time. For example, in October and November 2014, there was a peak of ~250 calls related to spice (specific substance names were rarely mentioned), the number equaling the total number for 2013 and most days comprised 5% and occasionally even 10% of all calls from hospitals. This peak coincided with what in the Swedish media was called the "spice epidemic" and may have been due to the introduction of new, potent, and more harmful SCRA variants on the Swedish online drug market (Bäckberg et al. 2017). At this time, they also started to be sold as pure substances instead of ready-for-use herbal smoking mixtures, increasing the risk for unintentional overdose. As the spice epidemic attracted a lot of attention in the media, this may have generated more calls. From the beginning of 2015, when 24 SCRAs were classified in Sweden, the number of PIC consultations related to spice showed a steady decline.

Another example of NPS variations over time relates to 3,4-methylenedioxypyrovalerone (MDPV), which was the first in a long series of pyrovalerone derivatives

sold as NPS. In Sweden, MDPV got widespread use after being regulated (in 2010) and was a common reason for consulting the PIC on severe intoxications in 2012–2013 (Beck et al. 2015). Interestingly, MDPV was reported to largely replace amphetamine on the illicit drug market for stimulants in some areas of the country (Lindeman et al. 2012). It should also be noted that MDPV became an established "street name" for other structural analogs that were introduced as replacement, e.g., alpha-pyrrolidinovalerophenone (α-PVP) and derivatives thereof (Beck et al. 2016). Since these structurally related substances share the same mechanism of action and pharmacological effects, drug users may have been unaware of the specific substance taken.

A third example relates to the designer fentanyls. When first launched on the Swedish NPS market in 2014 (Bäckberg et al. 2015b), these substances were hardly noticed at the PIC, possibly because cases with symptoms of opioid overdose (i.e., decreased consciousness, and respiratory depression) were already effectively treated with naloxone. Besides, routine drug testing for fentanyl is rarely performed. Nevertheless, the analysis of samples in the STRIDA project revealed use of many designer fentanyls in the country, typically in the form of nasal sprays (Bäckberg et al. 2015b; Helander et al. 2016). At the PIC, it was not until the summer of 2016 that one substance, acrylfentanyl (Helander et al. 2017b), was specifically noticed and the number of consultations exceeded the number of analytically confirmed intoxication cases in the STRIDA project. This also coincided with an increased media attention on fentanyl analogs due to many fatalities among young people and public warnings by the police (Helander et al. 2016).

3.4 Clinical Symptoms and Treatment of New Psychoactive Substances Intoxications

According to experiences at the Swedish PIC, the "first generation" SCRA products (e.g., herbal smoking blends containing JWH variants) (Marriott and Huffman 2008) typically gave relatively mild clinical signs and symptoms such as difficulties to concentrate, tachycardia, hypertension, and dilated pupils. Cases were characterized by a rapid (within 10 min of smoking) onset of effects, typically lasting for up to 6 h. If needed, treatment with a low dose of benzodiazepines was usually sufficient in such cases. Later, an increased risk of harm and adverse events associated with new SCRAs were noticed and suggested to reflect higher potency and toxicity of novel drug variants introduced in 2014 and beyond. In the STRIDA project, this was exemplified by analytically confirmed intoxications involving methyl-2-(1-(cyclohexylmethyl)-1H-indol-3-ylcarbonylamino)-3,3-dimethylbutanoate (MDMB-CHMICA) in 2014–2015, where patients presented with seizure episodes, deep unconsciousness, and respiratory depression (Bäckberg et al. 2017). These patients were transported to hospital by ambulance and police assistance was sometimes needed. Treatment with diazepam was used on ambulance arrival, during transport, and/or in hospital, and some needed sedation with propofol, intubation, and/or monitoring in the ICU.

Among several hundred analytically confirmed STRIDA cases involving MDPV, α-PVP, or structurally related variants, patients presented with a variety of often severe clinical signs and symptoms: agitation, delirium, hallucinations, excessive motor activity, seizures, tachycardia, hypertension, and/or hyperthermia. Pharmacological treatment primarily included use of benzodiazepines, haloperidol, and propofol. The greater potency of MDPV compared to what was normally their first choice of stimulant drug (i.e., amphetamine), initially resulting in overdosing, was suggested a likely reason for the increased incidence of severe intoxications causing major problems for emergency care (Beck et al. 2015; Lindeman et al. 2012).

In intoxications involving designer fentanyls, the clinical symptoms and standard treatment resembled those for fentanyl (Bäckberg et al. 2015b; Helander et al. 2016, 2017b). Accordingly, typical clinical features of opioid overdose were reported, such as decreased consciousness, respiratory depression, and miosis, and the opioid antidote naloxone was recommended to counter the potentially life-threatening adverse effects. However, for some derivatives that are considerably more potent opioid receptor agonists than the parent drug, the number of fatalities in Sweden exceeded the number of nonfatal intoxications in the STRIDA project (Helander et al. 2016) indicating overdosing problems and emphasizing the need for expanded availability of naloxone. Naloxone is currently in Sweden only available for paramedics and at hospitals.

Unexpected acute and late toxic effects of NPS opioids have also been encountered, including hearing loss, severe skin problems, and cataracts requiring surgery in the case of MT-45 (Bradley et al. 2016; Helander et al. 2017a).

3.5 Drug Materials in the STRIDA Project

The number of drug materials from STRIDA project cases submitted along with the biological samples increased by year from 7 in 2010, 16 in 2011, 37 in 2012, 42 in 2013, and 86 in 2014, to 118 in 2015. Of the total number of 306 drug materials received in the laboratory, 251 originating from 173 patients (1–10 materials from each case) were subjected to analysis (empty plastic bags and standardized sachets containing the patient's prescribed medicines were not). The drug materials (see Fig. 2 for examples) were encountered in the form of powders or crystalline (39%), tablets or capsules (32%), herbal materials (16%), liquids (8%), blotters (1%), and others (e.g., chewing gum and paper wad) (4%).

In 60% of drug materials, one or more NPS were analytically confirmed, but only about half (48%) had a label or marking indicating specific ingredients. Furthermore, in 30% of samples more than one psychoactive substance was detected, although some of the additional substances were present in only low or trace amounts and could hence constitute remnants of precursors or minor impurities from the production (e.g., despropionylfentanyl in the case of designer fentanyls) (Bäckberg et al. 2015b; Helander et al. 2016). In materials where no NPS were detected, the psychoactive contents were mainly classical drugs of abuse or medicines. Whether

Fig. 2 Examples of drug materials containing NPS obtained in the Swedish STRIDA project. The NPS materials were in the form of pure substances (powder and crystalline), spiked herbal smoking mixtures, blotters, tablets and capsules, and, in the case of fentanyl analogs, nasal sprays (liquids)

the pharmaceuticals were prescribed medicaments or retrieved illegally by the patients was unknown. In some materials, no psychoactive substance was detected.

More than 80 different NPS belonging to common classes of abused drugs (EMCDDA 2017) were detected in the drug materials such as aminoindans, arylalkylamines, arylcyclohexylamines, benzodiazepines, SCRAs, cathinones, tryptamines, opioids, phenethylamines, piperidines and pyrrolidines, plants and extracts, and "others" were represented, the most common being SCRAs and cathinones. About half of the substances were found only once, and those found several times were usually found within a limited time (<1 year). 4-Hydroxy-*N*-methyl-*N*-ethyltryptamine (4-HO-MET) (2010–2012), methoxetamine (2011, 2012, and 2014), *N*-[(2*S*)-1-amino-3-methyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl) methyl]-1*H*-indazole-3-carboxamide (AB-FUBINACA), ethylphenidate and α-PVP (2012–2014), and 3-methoxy-phencyclidine (2013–2015) are examples of NPS detected for several years, possibly because they gained a more widespread, established use among drug addicts and/or because they were initially not controlled (Bäckberg et al. 2018).

4 Conclusions

The STRIDA project has been demonstrated to serve as an effective EWS for NPS in Sweden by collecting data on the incidence and distribution, identification of adverse effects, and effective treatment of analytically confirmed acute intoxication cases relating to novel drugs of abuse presenting in ED and ICU. The STRIDA project has supported personnel at the PIC and thereby clinicians and other healthcare professionals all over the country. It contributed internationally through scientific

presentations and publications, in the knowledge and critical care of acute intoxications. They were caused by a wide range of NPS and these causes would otherwise likely been hidden. The results of the STRIDA project have also illustrated how regulations can drive the NPS market. An obvious reason for the large number of cases enrolled, and hence the success of the project, has been the opportunity to offer free drug testing. A necessity for successful analysis of NPS is the availability of sophisticated analytical instruments and ongoing method development in order to keep pace with the rapid turnover of new substances.

References

Al-Saffar Y, Stephanson NN, Beck O (2013) Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine-experience from the Swedish population. J Chromatogr B Analyt Technol Biomed Life Sci 930:112–120

Auwärter V, Dresen S, Weinmann W, Muller M, Putz M, Ferreiros N (2009) 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? J Mass Spectrom 44:832–837

Bäckberg M, Beck O, Hulten P, Rosengren-Holmberg J, Helander A (2014) Intoxications of the new psychoactive substance 5-(2-aminopropyl)indole (5-IT): a case series from the Swedish STRIDA project. Clin Toxicol (Phila) 52:618–624

Bäckberg M, Beck O, Helander A (2015a) Phencyclidine analog use in Sweden – intoxication cases involving 3-MeO-PCP and 4-MeO-PCP from the STRIDA project. Clin Toxicol (Phila) 53:856–864

Bäckberg M, Beck O, Jönsson KH, Helander A (2015b) Opioid intoxications involving butyrfentanyl, 4-fluorobutyrfentanyl, and fentanyl from the Swedish STRIDA project. Clin Toxicol (Phila) 53:609–617

Bäckberg M, Lindeman E, Beck O, Helander A (2015c) Characteristics of analytically confirmed 3-MMC-related intoxications from the Swedish STRIDA project. Clin Toxicol (Phila) 53:46–53

Bäckberg M, Westerbergh J, Beck O, Helander A (2016) Adverse events related to the new psychoactive substance 3-fluorophenmetrazine – results from the Swedish STRIDA project. Clin Toxicol (Phila) 54:819–825

Bäckberg M, Tworek L, Beck O, Helander A (2017) Analytically confirmed intoxications involving MDMB-CHMICA from the STRIDA project. J Med Toxicol 13:52–60

Bäckberg M, Jönsson KH, Beck O, Helander A (2018) Investigation of drug products received for analysis in the Swedish STRIDA project on new psychoactive substances. Drug Test Anal 10:340–349

Baron M, Elie M, Elie L (2011) An analysis of legal highs: do they contain what it says on the tin? Drug Test Anal 3:576–581

Beck O, Franzen L, Bäckberg M, Signell P, Helander A (2015) Intoxications involving MDPV in Sweden during 2010-2014: results from the STRIDA project. Clin Toxicol (Phila) 53:865–873

Beck O, Franzen L, Bäckberg M, Signell P, Helander A (2016) Toxicity evaluation of alpha-pyrrolidinovalerophenone (alpha-PVP): results from intoxication cases within the STRIDA project. Clin Toxicol (Phila) 54:568–575

Björnstad K, Beck O, Helander A (2009) A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. J Chromatogr B Analyt Technol Biomed Life Sci 877:1162–1168

Bradley M, Hasselblad A, Norlen L, Lapins J, Bäckberg M, Helander A (2016) Acute cutaneous symptom complex with subsequent cataract. The internet drug MT-45 may be the cause. Lakartidningen 113. DTAF

Davies S, Wood DM, Smith G, Button J, Ramsey J, Archer R, Holt DW, Dargan PI (2010) Purchasing 'legal highs' on the internet – is there consistency in what you get? QJM 103:489–493

Drug Enforcement Administration (2016) Counterfeit prescription pills containing fentanyls: a global threat. https://www.dea.gov/docs/Counterfeit%20Prescription%20Pills.pdf

EMCDDA (2009) Understanding the 'Spice' phenomenon. http://www.emcdda.europa.eu/attachements.cfm/att_80086_EN_EMCDDA_Understanding%20the%20%E2%80%98Spice%E2%80%99%20phenomenon_4Update%2020090813.pdf

EMCDDA (2017) European drug report 2017: trends and developments. http://www.emcdda.europa.eu/system/files/publications/4541/TDAT17001ENN.pdf

Gladden RM, Martinez P, Seth P (2016) Fentanyl law enforcement submissions and increases in synthetic opioid-involved overdose deaths – 27 states, 2013–2014. MMWR Morb Mortal Wkly Rep 65:837–843

Helander A, Bäckberg M (2016) New Psychoactive Substances (NPS) – the Hydra monster of recreational drugs. Clin Toxicol (Phila) 55(1):1–3

Helander A, Beck O, Hägerkvist R, Hulten P (2013) Identification of novel psychoactive drug use in Sweden based on laboratory analysis – initial experiences from the STRIDA project. Scand J Clin Lab Invest 73:400–406

Helander A, Bäckberg M, Beck O (2014a) MT-45, a new psychoactive substance associated with hearing loss and unconsciousness. Clin Toxicol (Phila) 52:901–904

Helander A, Bäckberg M, Hulten P, Al-Saffar Y, Beck O (2014b) Detection of new psychoactive substance use among emergency room patients: results from the Swedish STRIDA project. Forensic Sci Int 243:23–29

Helander A, Beck O, Bäckberg M (2015) Intoxications by the dissociative new psychoactive substances diphenidine and methoxphenidine. Clin Toxicol (Phila) 53:446–453

Helander A, Bäckberg M, Beck O (2016) Intoxications involving the fentanyl analogs acetylfentanyl, 4-methoxybutyrfentanyl and furanylfentanyl: results from the Swedish STRIDA project. Clin Toxicol (Phila) 54:324–332

Helander A, Bradley M, Hasselblad A, Norlen L, Vassilaki I, Bäckberg M, Lapins J (2017a) Acute skin and hair symptoms followed by severe, delayed eye complications in subjects using the synthetic opioid MT-45. Br J Dermatol 176:1021–1027

Helander A, Bäckberg M, Signell P, Beck O (2017b) Intoxications involving acrylfentanyl and other novel designer fentanyls – results from the Swedish STRIDA project. Clin Toxicol (Phila) 55:589–599

Johansson M, Fransson D, Rundlof T, Huynh NH, Arvidsson T (2014) A general analytical platform and strategy in search for illegal drugs. J Pharm Biomed Anal 100:215–229

Kamijo Y, Takai M, Fujita Y, Hirose Y, Iwasaki Y, Ishihara S, Yokoyama T, Yagi K, Sakamoto T (2014) A multicenter retrospective survey of poisoning after consumption of products containing synthetic chemicals in Japan. Intern Med 53:2439–2445

Lindeman E, Hulten P, Ström S, Enlund M, Al-Saffar Y, Helander A (2012) Ökat missbruk av Internetdrogen MDPV i Västmanland. Svåra förgiftningsfall har gett sjukvården stora problem. Lakartidningen 109:1954–1957

Lindeman E, Bäckberg M, Personne M, Helander A (2014) MT-45 – a dangerous and potentially ototoxic internet drug. Lakartidningen 111:1712–1715

Lindigkeit R, Boehme A, Eiserloh I, Luebbecke M, Wiggermann M, Ernst L, Beuerle T (2009) Spice: a never ending story? Forensic Sci Int 191:58–63

Marriott KS, Huffman JW (2008) Recent advances in the development of selective ligands for the cannabinoid CB(2) receptor. Curr Top Med Chem 8:187–204

Meyer MR, Pettersson Bergstrand M, Helander A, Beck O (2016) Identification of main human urinary metabolites of the designer nitrobenzodiazepines clonazolam, meclonazepam, and nifoxipam by nano-liquid chromatography-high-resolution mass spectrometry for drug testing purposes. Anal Bioanal Chem 408:3571–3591

Ojanperä I, Kriikku P, Vuori E (2016) Fatal toxicity index of medicinal drugs based on a comprehensive toxicology database. Int J Legal Med 130:1209–1216

Persson HE, Sjöberg GK, Haines JA, Pronczuk de Garbino J (1998) Poisoning severity score. Grading of acute poisoning. J Toxicol Clin Toxicol 36:205–213

Pettersson Bergstrand M, Helander A, Hansson T, Beck O (2017a) Detectability of designer benzodiazepines in CEDIA, EMIT II plus, HEIA, and KIMS II immunochemical screening assays. Drug Test Anal 9:640–645

Pettersson Bergstrand M, Meyer MR, Beck O, Helander A (2017b) Human urinary metabolic patterns of the designer benzodiazepines flubromazolam and pyrazolam studied by liquid chromatography-high resolution mass spectrometry. Drug Test Anal 10:496–506

Simonsen KW, Edvardsen HM, Thelander G, Ojanperä I, Thordardottir S, Andersen LV, Kriikku P, Vindenes V, Christoffersen D, Delaveris GJ, Frost J (2015) Fatal poisoning in drug addicts in the Nordic countries in 2012. Forensic Sci Int 248:172–180

Starmark JE, Stålhammar D, Holmgren E, Rosander B (1988) A comparison of the Glasgow Coma Scale and the Reaction Level Scale (RLS85). J Neurosurg 69:699–706

Stephanson NN, Signell P, Helander A, Beck O (2017) Use of LC-HRMS in full scan-XIC mode for multi-analyte urine drug testing – a step towards a 'black-box' solution? J Mass Spectrom 52:497–506

UNODC (2017) Fentanyl and its analogues – 50 years on. https://www.unodc.org/documents/scientific/Global_SMART_Update_17_web.pdf

WHO (2014) Community management of opioid overdose. http://apps.who.int/iris/bitstream/10665/137462/1/9789241548816_eng.pdf?ua=1&ua=1

Patterns of Acute Toxicity Associated with New Psychoactive Substances

Simon L. Hill and Paul I. Dargan

Contents

Abstract

This chapter begins by considering why it is important to understand the clinical patterns of acute toxicity associated with new psychoactive substances (NPS), the challenges associated with gathering these data, the sources of information available and the limitations of each. It describes the data triangulation approach that can be used to combine individual, each inherently limited, data sources to help build the picture of the pattern of acute non-fatal toxicity associated with NPS. The chapter illustrates the data triangulation approach by the use of clinical examples and aims to consider mechanism of action data in conjunction with clinical

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features to provide an overarching understanding of the clinical presentation. Examples of the most important individual and groups of NPS were identified using multimodal literature searching based on the most relevant terms. The chapter provides descriptive accounts that are a complete reference source on the patterns of acute toxicity.

Keywords

Cathinones · Drug of abuse · Fentanyls · New psychoactive substances · Novel psychoactive substances · NPS · Opioids · Overdose · SCRA · Synthetic cannabinoid receptor agonists · Toxicity · Toxidrome

Acronyms

α-PVP	1-Phenyl-2-(pyrrolidin-1-yl)pentan-1-one
2C-B	2-(4-Bromo-2,5-dimethoxyphenyl)ethan-1-amine
25I-NBOMe	2-(4-Iodo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethan-1-amine
4-MTA	1-[4-(Methylthio)phenyl]propan-2-amine
5F-NPB-22	Quinolin-8-yl-1-(5-fluoropentyl)-1*H*-indazole-3-carboxylate
5F-SDB-005	Naphthalen-1-yl 1-(5-fluoropentyl)-1*H*-indazole-3-carboxylate
AB-CHMINACA	*N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide
AH-7921	3,4-Dichloro-*N*-{[1-(dimethylamino)cyclohexyl]methyl}benzamide
AMB-FUBINACA	Methyl (2*S*)-2-({1-[(4-fluorophenyl)methyl]-1*H*-indazole-3-carbonyl}amino)-3-methylbutanoate
AMT	1-(1*H*-Indol-3-yl)propan-2-amine
DOB	1-(4-Bromo-2,5-dimethoxyphenyl)propan-2-amine
FUB-NPB-22	Quinolin-8-yl 1-(4-fluorobenzyl)-1*H*-indazole-3-carboxylate
FUB-PB-22	Quinolin-8-yl 1-[(4-fluorophenyl)methyl]-1*H*-indole-3-carboxylate
NM-2201	Naphthalen-1-yl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate
MDMB-CHMICA	Methyl (2*S*)-2-{[1-(cyclohexylmethyl)-1*H*-indole-3-carbonyl]amino}-3,3-dimethylbutanoate
MDPV	1-(1,3-Benzodioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one
PMMA	1-(4-Methoxyphenyl)-*N*-methylpropan-2-amine
U-47700	3,4-Dichloro-*N*-[(2*R*)-2-(dimethylamino)cyclohexyl]-*N*-methylbenzamide

1 Introduction

The dramatic increase in the availability of new psychoactive substances (NPS) across the globe has caused a sea-change in the epidemiology of drug misuse since the early parts of the twenty-first century (EMCDDA 2017; UNODC 2018). This change has been mediated, in part, by the emergence of online marketplaces for NPS (EMCDDA 2017; UNODC 2018). The result is a dynamic NPS landscape with important geographical and temporal variation in the substances misused and the clinical toxicity, or harms, associated. Currently more than 700 individual NPS are monitored as part of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) early warning system (EWS) with more than 70% having been identified only in the last 5 years (EMCDDA 2017).

Most NPS that enter global drug misuse markets have not undergone the mandatory preclinical and clinical testing and scrutiny required by regulatory authorities, such as the European Medicines Agency (EMA) or the US Food and Drug Administration (FDA), for marketing authorization of medicinal products. Consequently often little, if anything, is initially known about their pharmacodynamics, pharmaco- and toxicokinetics, clinical effects and perhaps most importantly their acute toxicity and the patterns of clinical adverse effects seen associated with their use. This challenges regulatory authorities considering the risk assessment of new NPS but also healthcare professionals dealing with individuals exposed to NPS and presenting to healthcare settings with acute toxicity. Accordingly there may be no information to support clinical assessment and management, such as determining the most appropriate location for clinical care (particularly whether critical care is necessary), guiding therapeutic interventions, predicting clinical deterioration, understanding the prognosis or anticipating duration of toxicity. A recent study has shown that healthcare professionals are less confident in managing acute toxicity associated with the use of NPS than acute toxicity associated with the use of established, classical recreational drugs (Wood et al. 2016).

Healthcare professionals face two additional challenges: It is uncommon that the particular NPS causing toxicity will be identified in a clinically meaningful timeframe. In a recent outbreak of NPS toxicity, identification in 17 days was well regarded (Adams et al. 2017). Sometimes users are unaware of the NPS they have taken as they are contained within branded products. Manufacturing quality control (QC) is commonly poor meaning significant heterogeneity exists in the type and concentration of NPS in each sample and in the presence of by-products of chemical synthesis, adulterants and other impurities (Brunt et al. 2017; Brandt et al. 2011). Analytical tests to confirm NPS in biological samples are not often available in acute settings and, those that are, typically test only for classical drugs of misuse. Such immunoassays are largely unable to detect NPS (Wagmann and Maurer 2018). Mass spectrometry-based procedures are capable of NPS identification although are far less available and much more expensive and require sophisticated data handling and interpretation, and results can take many days or weeks (Wagmann and Maurer 2018). Therefore, results are not available at the point of acute care decision-making. In addition, it is common for multiple NPS to be used simultaneously or to be used

with classical recreational drugs, making it unclear which individual NPS, or particular combinations of NPS/classical drug(s), are responsible for the clinical features (Helander et al. 2014; Cole et al. 2015; Thomas et al. 2016).

In practice – not just for NPS but also for classical drugs – clinicians identify a clinical toxidrome for each patient based on the clinical features at presentation. Different classes of NPS are associated with different toxidromes, for example, stimulant, depressant or dissociative. This approach allows management decisions to be made in the absence of specific NPS identification. It is therefore essential that the patterns of acute clinical toxicity associated with NPS are clearly described and can be recognized by clinicians, linked to consideration of probable pharmacodynamics actions and used to inform potential therapeutic strategies. Unfortunately, data on acute toxicity are not routinely collected by international bodies such as the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) or the United Nations Office on Drugs and Crime (Heyerdahl et al. 2014). One reason for this is that national and international hospital admission coding systems are not reliable for characterizing acute recreational drug toxicity, particularly that relating to NPS (Wood et al. 2014). It is necessary instead to combine data from a number of sources to provide early insight into the acute toxicity associated with the use of NPS. The approach has been called data triangulation, and although each source has individual limitations, the process of data triangulation helps to decrease the impact of the limitations by drawing on multiple sources (Sedefov et al. 2013). Table 1 shows some of the sources used, the data they may impart and their inherent limitations.

2 Methods

This chapter is not intended as a systematic review of every pattern of acute toxicity associated with every NPS. Instead, examples of the most important individual and groups of NPS were identified using multimodal literature searching based on the most relevant terms. Additionally back searching of the references in identified articles and forward searching of citations of those identified articles were undertaken.

3 Synthetic Cannabinoid Receptor Agonists (SCRAs)

Synthetic cannabinoid receptor agonists (SCRAs) are the largest group of NPS monitored by the EMCDDA with 169 substances detected since 2008, and they are becoming increasingly chemically diverse (EMCDDA 2017). SCRAs account for 29% of all NPS law enforcement seizures reported to the EU EWS (EMCDDA 2017). Most SCRAs are full CB_1 and CB_2 receptor agonists with a higher potency than Δ^9-trans-tetrahydrocannabinol (THC), with increasing CB_1 receptor potency being seen in the more recent third-generation SCRAs (Wiley et al. 2013, 2015; Huffman et al. 2005). The psychoactive effects of SCRAs are associated with their

Table 1 Sources of data that may inform understanding of the patterns of acute toxicity associated with new psychoactive substances

Data source	Analytical (A) or nonanalytical (NA)	Strengths	Limitations
Postmortem	A	Highlight most severe toxicity	Slow process; may take more than 1 year
			Often limited clinical data available
Toxicovigilance programmes	A	Robust data, usually standardized collection proforma; can show trends over time	Few exist, incomplete global coverage so may not reflect practice in non-participating locations
Case series	A	Usually detailed clinical data	Specialist centres may not reflect usual practice elsewhere
			Publication bias
			May not include analytical confirmation of the drug (s) involved
Case reports	A	Usually very detailed clinical data	May be an exception rather than rule
			Publication bias
			May not include analytical confirmation of the drug (s) involved
User forums	NA	Earliest information	Non-healthcare reporting, accuracy unclear of clinical features and substances involved
Surveys	NA	Early information	Self-selected population, non-healthcare reporting
		Usually correlate to analytically confirmed series	
Hospital admissions data	NA	High-volume data	Poor granularity, usually unable to identify individual or even classes of drugs
Poison centres	NA/A	High number of cases and focused on cases causing harm	Rates of detection may decline with familiarity with the substances involved
		Aggregate data correlate well with analytically confirmed series	Data depend upon voluntary reporting
		May detect new and unfamiliar exposures	Often lack analytical confirmation and may not discern which symptoms to attribute to a given substance in cases of poly-drug exposure
		Can look at trends and changes over time	Novel adverse events and events involving new NPS are more likely to be reported or published in the medical literature

CB_1 receptor affinities (Wiley et al. 1995). SCRAs are typically smoked although they can be vaped in e-cigarettes as either liquid or herbal forms or used as powder (Blundell et al. 2017).

The pattern of acute clinical toxicity associated with SCRA use as reported in the published literature up to 31st December 2014 was described in a systematic review using a data triangulation approach (Tait et al. 2016). Using both self-reported and analytically confirmed exposures, largely poison centre data, the pattern of acute toxicity aggregated from over 4,000 cases was described. The prototypical presentation is a young male (59–100%) with tachycardia (37–77%), agitation (16–41%) and nausea (13–94%). Severe features reported included chest pain, myocardial infarction and stroke; acute kidney injury (AKI); seizures and rarely status epilepticus; acute psychosis, panic, hallucination and paranoia; and death attributed either directly (arrhythmia, seizure, multi-organ failure) or indirectly (hypothermia, trauma, self-harm) to SCRA.

Case series of SCRA intoxication with analytical confirmation of exposure align closely with the systematic review. For example, Abouchedid et al. report an emergency department (ED) series of patients with acute recreational drug toxicity in which synthetic cannabinoids were detected in 18 (10%) patients (Abouchedid et al. 2017). The most frequently observed clinical features were tachycardia ($n = 5$, 28%), chest pain ($n = 1$, 6%), seizures ($n = 4$, 22%), coma ($n = 1$, 6%), agitation ($n = 4$, 22%) and psychosis ($n = 1$, 6%). As expected, multiple NPS were present in most cases with only two cases of single SCRA exposure.

In the Global Drug Survey, published in 2015, 2,176 people reported lifetime use of synthetic cannabinoid receptor agonists (Winstock et al. 2015). Users of SCRAs were more likely to report ever having experienced panic and anxiety (81.0% vs. 59.5%), paranoia (61.9% vs. 35.1%), agitation (47.1% vs. 21.6%), chest pain (33.3% vs. 19.6%), visual hallucinations (33.3% vs. 16.2%), seizures (19.0% vs. 8.1%) and accidents (14.3% vs. 8.1%) compared to 19,024 users of cannabis.

SCRAs have evolved over time, aiming to evade legislative control and perhaps to also enhance desired recreational effects, and in doing so have typically increased in potency (Adams et al. 2017). In the UK, more recent iterations are called 'third-generation' SCRAs (ACMD 2014), and additional, probably greater, patterns of toxicity have been reported for some of these, especially if one considers the potential for overdosing. Hill et al. reported seven cases of toxicity associated with MDMB-CHMICA, including three cases of isolated exposure (Hill et al. 2016). The clinical toxicity included reduction in consciousness and respiratory depression with type II respiratory failure, hitherto unreported, and bradycardia. These features are not part of the stimulant/sympathomimetic toxidrome (unless explained by post ictal state, cardiovascular collapse, central nervous system (CNS) lesion) (Bäckberg et al. 2015b). Hermanns-Clausen et al. subsequently also reported respiratory depression, in 61% of 44 cases of MDMB-CHMICA ($n = 24$) or AB-CHMINACA ($n = 20$) toxicity presenting to the ED (Hermanns-Clausen et al. 2017). The authors compared this cohort with a previous cohort of patients with analytically confirmed toxicity due to an earlier generation of aminoalkylindoles. The more recent SCRAs were associated with more severe toxicity as determined by the poison severity score

(PSS) (21% vs. 3%, respectively) and a greater likelihood of prolonged toxicity (>1 day and up to 3 weeks) (34% vs. 3%, respectively). Summarizing published MDMB-CHMICA cases, the authors report an overall increase in the frequency of sedation ($n = 18, 55\%$), seizures ($n = 11, 33\%$) and agitation ($n = 8, 24\%$) compared to earlier generations of SCRAs (Hermanns-Clausen et al. 2017). Most of these patients tested positive for other drugs ($n = 18, 55\%$). Another recently identified sub-group of SCRAs, the indole and indazole carboxylates (such as FUB-NPB-22, 5F-NPB-22, 5F-SDB-005, FUB-PB-22, NM-2201), were associated with similar severity of clinical features in 17 analytically confirmed cases, specifically confusion, agitation or aggression ($n = 14$; 82%), reduced consciousness ($n = 13$; 76%), acidosis ($n = 8$; 47%), hallucinations and paranoid features ($n = 7$; 41%), tachycardia ($n = 6$; 35%), hypertension ($n = 5$; 29%), raised creatine kinase ($n = 4$; 24%) and seizures ($n = 2$; 12%) (Hill et al. 2018). A cluster of severe cases of SCRA toxicity occurred in New York, USA, described by the media as a 'zombie outbreak' due to the profound CNS depression seen (Adams et al. 2017). The responsible SCRA identified in products was AMB-FUBINACA, but the toxidrome was unusually characterized by the absence of tachycardia, arrhythmia, seizures, hyperthermia, cardiotoxicity and acute kidney injury (Adams et al. 2017).

Acute kidney injury was initially recognized as an infrequent part of the pattern of acute SCRA toxicity following an alert in the USA in 2012 (CDC 2013). Sixteen cases were reported with peak serum creatinine concentrations ranging from 3.3 to 21.0 mg/dL (median: 6.7 mg/dL; normal 0.6–1.3 mg/dL) that occurred 1–6 days after symptom onset (median: 3 days). Urinalysis available for 15 patients showed proteinuria ($n = 8$), casts ($n = 5$), white blood cells ($n = 9$) and red blood cells ($n = 8$). Renal ultrasonography was performed in 12 patients, 9 reported a nonspecific increase in renal cortical echogenicity, and none had hydronephrosis. Eight patients underwent renal biopsy that demonstrated acute tubular injury ($n = 8$) or acute interstitial nephritis ($n = 3$). Kidney function recovery was apparent within 3 days of creatinine peak in most patients. However, 5 of the 16 patients required haemodialysis, and 4 patients received corticosteroids; none died. XLR-11 and/or XLR-11 metabolites were found in five of the seven cases for whom clinical specimens were available, although causality was not proven. Ukaigwe et al. reported that prerenal injury may follow profound nausea and vomiting sometimes seen with SCRA use (perhaps akin to the cannabis hyperemesis syndrome) and rhabdomyolysis has been reported with SCRAs and may also contribute to acute kidney injury (Ukaigwe et al. 2014). There is a case report of thrombotic microangiopathy associated with SCRA use leading to acute renal failure and transplant and additionally a significant decline of the left ventricular ejection fraction (Karass et al. 2017).

There is a single report of acute disseminated encephalomyelitis (ADEM) secondary to SCRA use (NM-2201) (Samra et al. 2017). A 25-year-old man presented with agitation, double incontinence and left-sided incoordination. Over 48 h, he developed aphasia, generalized hypertonia, hyperreflexia and dense left hemiparesis that progressed to profuse diaphoresis, pyrexia, tachycardia, hypertension and a possible seizure necessitating admission to the intensive care unit. MRI brain

demonstrated multifocal hyperintense contrast-enhancing lesions, which was suggestive of ADEM. He made a slow but significant recovery after a course of intravenous methylprednisolone, intravenous immunoglobulins and oral steroids and was nearly back to his baseline 9 months later.

In summary, the SCRA toxidrome is characterized by:

1. A history of exposure by smoking or less frequently vaping
2. Cardiovascular dysfunction (typically tachycardia and hypertension but less commonly bradycardia, acute coronary syndrome, arrhythmias or other vascular complications)
3. Neuropsychiatric dysfunction (unexplained reduced consciousness including type II respiratory failure and acidaemia and sometimes seizures; often profound agitation and panic, psychosis, hallucinations and violence)
4. Gastrointestinal dysfunction (nausea, vomiting, sometimes hyperemesis)
5. Renal dysfunction (acute kidney injury by multiple mechanisms)

4 Synthetic Cathinones

Synthetic cathinones are psychostimulant drugs, related to and derived from classical drugs of misuse such as amphetamine and cathinone, the active constituent of Khat (*Catha edulis*) (EMCDDA 2017). Not all cathinones show identical pharmacological properties, and important differences exist within the group in the patterns of clinical toxicity they cause.

They are usually sold in powder or crystalline form, in some cases under generalized names – such as 'bath salts' (in the USA). Cathinones account for 33% of law enforcement seizures of NPS in the European Union (EU) with seized materials amounting to more than 1.8 tons (EMCDDA 2017). They are the second largest group by number of NPS monitored by the EU EWS. They also led to acute ED presentations as shown by data from the Euro-DEN study where cathinones were the most commonly reported class of NPS ($n = 378$, 78%) of which mephedrone was the most frequent (64% of cathinones) followed by methedrone (24%) and MDPV (5%) ((EURO-DEN) 2015).

Like other psychostimulants, cathinone NPS are monoamine uptake inhibitors, but many also induce transporter-mediated monoamine efflux with weak or no activity at pre- or postsynaptic receptors (Simmler and Liechti 2017). Cathinones exhibit clinically relevant differences presumably associated with relative selectivity and potency at serotonin, dopamine and, probably most importantly, noradrenaline reuptake transporters. In some cases hybrid mechanisms of action have been identified with, for example, dopamine reuptake inhibition and concurrent serotonin release (Blough et al. 2014; Saha et al. 2015). Accordingly, the group can be classified in a clinically useful way, based on the similarity of their patterns of acute toxicity compared to well-described classical drugs of misuse, that aligns closely with differences in their pharmacodynamic actions, specifically differences

in selectivity and potency of inhibition (and transporter-mediated release (Baumann et al. 2013a)) at the dopamine (DAT) and serotonin (SERT) reuptake transporters expressed as DAT:SERT ratio. Based on the approach taken by Baumann et al. (2000), Simmler et al. proposed four sub-groups of NPS cathinones according to monoamine transporter uptake inhibition data collected in vitro (Simmler et al. 2013):

1. MDMA-like (DAT:SERT <1)
2. Mixed MDMA-cocaine-like (DAT:SERT ~1)
3. Methamphetamine-like (DAT:SERT ~5–100)
4. Methylphenidate-like (DAT:SERT >100)

4.1 Mephedrone

For many, NPS came to the fore following the emergence of mephedrone. In the UK, this occurred in 2009 (James et al. 2011; Kamour et al. 2014). Mephedrone can be regarded as the archetypal ring-substituted cathinone, and the pattern of acute toxicity has been well described (discussed below). It belongs in the mixed MDMA-cocaine-like sub-group of cathinones with a DAT:SERT ratio in vitro of 1.4 (95% CI 0.9–2.4) (Simmler et al. 2013). In this section, we describe the clinical toxicity of mephedrone and subsequently consider other cathinones by way of comparison with it.

In 2010, Wood et al. reported the first analytically confirmed case series of mephedrone toxicity in seven male patients, four without other co-ingestion identified (two patients had co-ingested cocaine and one had two additional cathinones) (Wood et al. 2010). The route of exposure was nasal insufflation or oral ingestion. The authors describe a sympathomimetic toxidrome characterized by agitation ($n = 4$), tachycardia ($n = 5$), hypertension ($n = 3$), chest pain ($n = 2$) and seizures ($n = 1$). Hyponatraemia and elevated creatine kinase were noted in one patient each. No patients had hyperthermia.

Aggregated data from the UK National Poisons Information Service included information from telephone enquiries about 131 patients presenting to healthcare facilities with adverse effects related to reported mephedrone use, either alone or with alcohol (James et al. 2011). These data also described a sympathomimetic toxidrome with common clinical features reported including agitation or aggression (24%), tachycardia (22%), confusion or psychosis (14%), chest pain (13%), nausea (11%), palpitations (11%), peripheral vasoconstriction (8%) and headache (5%). Convulsions were reported in four cases (3%). Forty-five and 30% of cases described a duration of symptoms greater than 24 and 48 h, respectively. In the USA, the severe end of this toxidrome is sometimes called ‘excited delirium’ (Penders et al. 2012).

In a randomized, double-blind, crossover, placebo-controlled trial, 12 healthy male volunteers were given mephedrone 200 mg orally and compared with 100 mg MDMA (Papaseit Fontanet et al. 2016). Mephedrone produced a significant increase

in systolic and diastolic blood pressure, heart rate, oral temperature and pupillary diameter. It elicited stimulant-like effects, euphoria and well-being and induced mild changes in perceptions (not hallucination or psychosis) comparable with MDMA administration. There were no serious adverse events. Onset of effect was at 30–45 min, maximal plasma concentration (mean 134 ng/mL) occurred at 1.25 h, and the elimination half-life for mephedrone was 2.15 h (Papaseit Fontanet et al. 2016). The authors stated that mephedrone in this context produced similar clinical effects to MDMA which is in keeping with the pharmacodynamics classification suggested by the Simmler et al. (2013).

Online survey data, of self-identified recreational drug users associated with the (primarily UK) dance music scene, similarly reported a sympathomimetic toxidrome characterized by excessive sweating, palpitations, nausea, headache, increased sex drive and cold blue fingers (Winstock et al. 2011).

Papaseit et al. have recently summarized user reports of mephedrone effects as (Papaseit et al. 2017): 'Pleasurable effects such as euphoria, increased energy, mood enhancement, talkativeness, increase music sensitivity, empathy, sociability, sensory enhancement, moderate sexual arousal, and perceptual distortions;

Undesirable effects include jaw clenching, bruxism, body sweats, palpitations, anxiety, tremor in extremities, blurred vision, shortness of breath, headache, cold or numb extremities, nausea and vomiting, agitation, anxiety, aggressiveness, paranoia, and panic, most of which are slight or moderate and do not require medical assistance;

Post-drug recovery effects include craving, decreased appetite, lack of motivation, paranoia, insomnia, and irritability, all of which ameliorate after a few days ("feeling normal" after ~4 days)'.

There are a number of additional serious features of toxicity due to mephedrone reported without analytical confirmation and hence requiring cautious interpretation. A case report of a 25-year-old female who presented with recurrent seizures after reported mephedrone use that was associated with characteristic magnetic resonance imaging features of posterior reversible encephalopathy syndrome (PRES) (Omer and Doherty 2011): A 33-year-old male with methaemoglobinaemia of >25% (Ahmed et al. 2010). Cardiomyopathy: A 27-year-old presented with dilated cardiomyopathy (LVEF 15–20% with global hypokinesia), following reported (but not analytically confirmed) mephedrone and 3,4-methylenedioxypyrovalerone (MDPV) intravenous and intranasal use. He had normal coronary angiography and viral serology (Sivagnanam et al. 2013).

4.2 Other Cathinones and How They Differ from the Mephedrone Archetype

4.2.1 Methylphenidate-Like (DAT:SERT >100): Pyrovalerone-Like Cathinones MDPV and α-PVP

The pyrovalerone-based cathinones MDPV and α-PVP are examples of highly potent NET and DAT reuptake inhibitors with lower potency at SERT; consequently

they have DAT:SERT ratios above 100. They are not monoamine releasers (substrates) at monoamine transporters (Simmler et al. 2013; Baumann et al. 2013b). The pattern of acute toxicity in 30 cases of analytically confirmed MDPV mono-intoxication and 163 cases of MDPV plus co-ingested substances has been reported as part of the STRIDA project (Beck et al. 2015). In mono-intoxications a sympathomimetic (psychostimulant) toxidrome characterized by tachycardia (70%), agitation (63%), delirium (27%) and hypertension (30%) has been described. Hyperthermia (>39°C) was less common in mono-intoxications than in the cohort with co-ingestion of other substances (7 vs. 10%, respectively) consistent with preclinical studies and known pharmacodynamics (i.e. less serotoninergic) (Miner et al. 2017) although there have been case reports of MDPV-induced hyperthermia (Borek and Holstege 2012). MDPV concentrations are not collected at fixed time points post-ingestion in the STRIDA methodology, but serum concentrations ranged between 1.0 and 1,509 ng/mL (mean 63.6, median 20) and in urine between 1.0 and 81,000 ng/mL (mean 3,880, median 1,160). MDPV/creatinine ratio ranged between 0.10 and 2,480 ng/mmol (mean 247, median 92.6) (Beck et al. 2015).

Additionally the STRIDA group have reported on 13 analytically confirmed α-PVP mono-intoxications describing an almost identical pattern of acute toxicity and with hyperthermia being relatively uncommon (1 case) (Beck et al. 2016) with mean and median serum concentrations of 125 and 64 ng/mL for α-PVP. Other case series report the same pattern of acute toxicity (Umebachi et al. 2016; Patel et al. 2017). In a series of 348 patients with recreational drug-related psychosis from the Euro-DEN group, psychosis was much more common amongst presentations involving MDPV (27.3%) than those involving mephedrone (5.7%) or methedrone (3.3%) (Vallersnes et al. 2016) consistent with the differences in their ability to increase dopaminergic tone (Baumann et al. 2013b; Simmler et al. 2013).

4.2.2 MDMA-Like (DAT:SERT < 1): Methedrone

Perhaps fortunately there are very few NPS cathinones from this sub-group that have, as yet, been identified in physical or biological samples. This group have low DAT:SERT ratios <1. Methedrone, for example, has a ratio of 0.14 (95% CI 0.04–0.46), comparable to MDMA (0.08) and the *para*-methoxy- and methylthio-substituted amphetamines, such as PMMA (0.04) and 4-MTA (0.02), respectively (Simmler et al. 2013). Such substances seem to be commonly associated with severe toxicity, characterized by hyperthermia and multi-organ failure that may be due to a severe serotonin syndrome. Supporting such concerns there have been two fatal intoxications due to methedrone, one of which was characterized by ante-mortem hyperthermia (Wikström et al. 2010). However, methedrone is less potent at NET inhibition than the serotoninergic substituted amphetamines discussed above (Simmler et al. 2013).

5 *N*-[(2-Methoxyphenyl)Methyl]-Substituted Phenethylamines

A new class of NPS with sympathomimetic and hallucinogenic patterns of toxicity, known as *N*-benzylphenethylamines (NBOMe compounds), have been available since 2010 (Halberstadt 2017). The phenethylamine structure is related to other hallucinogenic phenethylamines and amphetamines (e.g. 2C-B and DOB) that also carry two methoxy groups at the 2 and 5 positions of the phenyl ring (see Hill and Thomas 2011 for chemical classification of the phenethylamine group) (Hill and Thomas 2011). These groups confer hallucinogenic activity via partial $5HT_{2A}$ agonism (Halberstadt 2017; Rickli et al. 2015). The *N*-benzyl substitution of hallucinogenic phenethylamines leads to increased affinity and potency (Rickli et al. 2015; Braden et al. 2006; Nichols et al. 2015), and it is this increase in potency that led to circulation of these substances on small blotters where exposures included misrepresented lysergic acid diethylamide products (Halberstadt 2017).

Twenty-five analytically unconfirmed cases of NBOMe ingestion were reported to the Texas poison control centre in 2012 and 2013 (Forrester 2014). Tachycardia (52.0%), agitation (48.0%) and hallucinations (32.0%) were most commonly noted, with 2 (8.0%) fatal outcomes (Forrester 2014).

Expanding work by Suzuki et al. (2015) and Wood et al. (2015), Halberstadt has summarized 51 published cases of analytically confirmed NBOMe toxicity and described 2 patterns of acute toxicity: mild toxicity and moderate to severe toxicity (Halberstadt 2017). In summary, the features in mild cases ($n = 24$, 47%) are those of a sympathomimetic toxidrome with added hallucinations, which is generally short lived and resolves spontaneously. Typical features are tachycardia, hypertension, mydriasis, tachypnoea and diaphoresis with agitation, confusion and hallucination. The pattern of acute severe NBOMe toxicity, seen in 23/51 cases (45%), includes seizures (87% of severe cases), rhabdomyolysis/raised creatine kinase (65%), metabolic acidosis (35%), acute kidney injury (43%), hyperthermia (52%) and multiorgan failure, coma and death (26%).

Making sense of the pathophysiology in severe NBOMe toxicity is challenging, and multiple processes are probably concurrently in operation. Certainly there is partial $5HT_{2A}$ agonism, and serotonin toxicity has been suggested as the culprit mechanism (although the diagnostic criteria have infrequently been met) (Halberstadt 2017). Monoamine reuptake inhibition or release is not a feature (Rickli et al. 2015). The relative roles of adrenoceptors are unknown. The aetiology of acute kidney injury is likely multifactorial including vasoconstriction, rhabdomyolysis and hypovolaemia.

6 AMT

α-Methyltryptamine, similar to some of the NBOMe compounds, shows both psychostimulant and hallucinogenic properties and can be considered an indole analogue of amphetamine. In vitro it is a triple catecholamine reuptake inhibitor

(SERT>NET > DAT) and releaser (Nagai et al. 2007). Furthermore, it is a potent and efficacious $5HT_{2A}$ receptor agonist (Arunotayanun et al. 2013). The UK National Poisons Information Service reported the pattern of acute toxicity of AMT from 63 telephone enquiries (analytically unconfirmed) from 2011 to 2013 and compared the pattern to that of mephedrone (Kamour et al. 2014). The pattern of toxicity was stimulant (65%), acute mental health disturbance (65%) with hallucinations in 24%, gastrointestinal upset (20%), fever/abnormal sweating (20%) and seizures (16%) (Kamour et al. 2014). In comparison to mephedrone, severe features, either as individual symptoms or expressed via the poison severity score, were more likely to occur with AMT (e.g. seizures: 16% vs. 2%; OR, 9.35; 95% CI 3.26–24.18).

7 Dissociatives

Methoxetamine, a derivative of the dissociative anaesthetic ketamine, was identified in the UK in 2010 (Morris and Wallach 2014). Methoxetamine acts as a non-competitive *N*-methyl-D-aspartate receptor antagonist (Roth et al. 2013). Cases of analytically confirmed toxicity described patterns of acute toxicity as psychostimulant and a ketamine-like dissociative state and also describe cerebellar dysfunction (Shields et al. 2012; Wood et al. 2012; Hofer et al. 2012). The latter is important as this is not a common feature of other NPS or classical recreational drugs.

Poison centre data from the UK, without analytical confirmation of exposure, confirmed a similar pattern of acute toxicity in 47 telephone enquiries such as stimulant (tachycardia, hypertension, mydriasis, palpitation, increased sweating) in 36%, acute mental health disturbance (agitation, confusion, euphoria, aggression, hallucination, paranoia, hysteria, manic reaction, psychosis) in 43%, dissociative (catatonia, dystonia, hypertonia, tetany) in 11%, cerebellar dysfunction (nystagmus, tremor) in 6%, reduced consciousness (reduced conscious level, stupor, somnolence, coma) in 17% (9 to 30) and seizures in 2% (Hill et al. 2014). A number of other NPS with dissociative toxidrome have been identified and are reviewed in detail by Morris and Wallach (2014).

8 New Synthetic Opioids

The appearance of NPS opioids is of particular concern because mortality is traditionally associated with drug misuse of classical opioids such as heroin, methadone and oxycodone (ONS 2017). These concerns have been realized, for example, translating into a substantial increase in an opioid-related death rate in the USA (Rudd et al. 2016). Morbidity and mortality are mediated by μ-opioid receptor agonism, and the acute pattern of toxicity is well known with reduced consciousness and respiratory depression (and meiosis, non-cardiogenic pulmonary oedema);

management with non-selective competitive opioid receptor antagonists, such as naloxone, is in widespread use.

There are around 40 novel opioids currently monitored by the EMCDDA with the majority being fentanyl derivatives (EMCDDA 2017). The synthetic μ-opioid receptor agonist U-47700, originally developed and investigated by the Upjohn pharmaceutical company in the 1970s, has 7.5 times more analgesic potency than morphine in vivo and has caused acute harms (Domanski et al. 2017) and 23 deaths. Similar reports of typical opioid toxicity and fatalities have been reported for AH-7921, a structural isomer of U-47700 originally developed by the Allen and Hanburys pharmaceutical company (Coppola and Mondola 2015; Katselou et al. 2015. In each case the pattern of acute toxicity mirrors that of the classical opioid toxidrome, and, where used, naloxone (2 mg, iv) was effective in reversing toxicity (Jones et al. 2017; Schneir et al. 2017).

Fentanyl is a synthetic, full μ-opioid receptor agonist with analgesic potency around 60- to 100-fold that of morphine in animal (Armenian et al. 2017) and human studies (Pereira et al. 2001). Fentanyl misuse has increased in recent years (EMCDDA 2017), and deaths are relatively common. Eighteen fentanyl derivatives have been detected in the EU since 2009 and 8 since 2016 (EMCDDA 2017). Many are substantially more potent analgesics than morphine and fentanyl in vivo, although data are not available on the in vitro potency of most of these fentanyls and how this in vivo data translates into risk assessment in the acutely poisoned patient is unclear (Armenian et al. 2017).

The STRIDA group have published analytically confirmed reports describing the patterns of acute toxicity related to the novel fentanyl analogues butyrfentanyl, 4-fluorobutyrfentanyl, acetylfentanyl, 4-methoxybutyrfentanyl, 2-furanylfentanyl, acrylfentanyl, 4-chloroisobutyrfentanyl, 4-fluoroisobutyrfentanyl, 2-tetrahydrofuran fentanyl and cyclopentylfentanyl (Helander et al. 2017; Bäckberg et al. 2015a; Helander et al. 2016). In all cases the typical opioid toxidrome is present, along with tachycardia and hypertension in some cases. Theoretically the greater potency and higher affinity of fentanyl analogues could require an increased dose of the competitive antidote naloxone; however, where used, the doses of naloxone required were found to be similar to those for classical opioids (Bäckberg et al. 2015a; Helander et al. 2016, 2017). Conversely, during a 2006 fentanyl outbreak, naloxone was given in 26/55 (47.3%) of ED visits, and doses ranged from 0.4 to 12 mg, with six cases requiring naloxone doses of at least 6 mg to reverse respiratory depression (Schumann et al. 2008). A 0.4 mg dose was only effective in 15% of cases requiring naloxone. It is likely given the higher potency and longer duration of action of these novel fentanyls that higher and/or repeated doses of naloxone will be required in the management of acute toxicity. One of the challenges to the clinician managing patients is that they will not know whether the patient has taken a classical or novel opioid, and therefore the current practice of giving small boluses of naloxone titrated against clinical response remains appropriate (Clarke et al. 2005).

Cole et al. described the case of an 18-year-old boy who developed hypoxic respiratory failure and diffuse alveolar haemorrhage after insufflating what he

thought was acetylfentanyl but after analysis was found to be butyrfentanyl (Cole et al. 2015). More recently carfentanyl, so far the most potent of the fentanyl analogues, has been identified associated with deaths in the USA and the EU (Hikin et al. 2018; Elliott and Hernandez Lopez 2018). A non-fatal analytically confirmed case report exists of a 16-year-old male found unconscious (Glasgow Coma Scale = 3), hypotensive (71/58 mmHg), tachycardic (126 bpm) and hypopneic and cyanotic (peripheral oxygen saturation 70%, no signs of pulmonary oedema) (Müller et al. 2018). In addition to the typical toxidrome, the response to naloxone (dose not reported) was also said to be in accordance with opioid intoxication. Conversely there are anecdotal reports of carfentanyl toxicity requiring many doses of naloxone to reverse the opioid effects, up to 18 mg in one report (Gussow 2016).

9 Conclusions

The patterns of acute toxicity associated with new psychoactive substances must be clearly described and understood in order to facilitate safe patient care and to inform regulatory decisions around legislative control. In this chapter, we have shown that it is necessary to use a data triangulation approach to synthesize multiple, individually limited, sources of information to inform a broad and clear understanding of the acute pattern of toxicity associated with individual substances and with groups of NPS. We have documented clearly the acute patterns of toxicity associated with synthetic cannabinoid receptor agonists, synthetic cathinones, hallucinogenic phenethylamine derivatives, dissociative NPS and novel synthetic opioids. Where possible it is helpful to associate pharmacodynamics and pharmacokinetic basic science research to question the plausibility of clinical findings. Some NPS are inherently toxic and can cause harm and death. We often know little about them when they first emerge into drug misuse markets. Here we have shown the value of national toxicovigilance systems through poison control centres and especially those linked to analytical confirmation of exposure, such as the STRIDA project in Sweden and the IONA project in the UK, in providing key information for regulators and clinicians.

References

European Drug Emergencies Network (2015) Final report of the European Drug Emergencies Network (Euro-DEN) March 2015

Abouchedid R, Hudson S, Thurtle N, Yamamoto T, Ho JH, Bailey G, Wood M, Sadones N, Stove CP, Dines A, Archer JRH, Wood DM, Dargan PI (2017) Analytical confirmation of synthetic cannabinoids in a cohort of 179 presentations with acute recreational drug toxicity to an Emergency Department in London, UK in the first half of 2015. Clin Toxicol (Phila) 55:338–345

Advisory Council on the Misuse Drugs (2014) 'Third generation' synthetic cannabinoids. Report to the Minister for Crime Prevention, which recommends a revised generic description of 'third

generation' synthetic cannabinoids. https://www.gov.uk/government/publications/third-generation-synthetic-cannabinoids

Adams AJ, Banister SD, Irizarry L, Trecki J, Schwartz M, Gerona R (2017) "Zombie" outbreak caused by the synthetic cannabinoid AMB-FUBINACA in New York. N Engl J Med 376:235–242

Ahmed N, Hoy BPS, Mcinerney J (2010) Methaemoglobinaemia due to mephedrone ('snow'). BMJ Case Rep. bcr0420102879

Armenian P, Vo KT, Barr-Walker J, Lynch KL (2017) Fentanyl, fentanyl analogs and novel synthetic opioids: a comprehensive review. Neuropharmacology pii: S0028-3908(17)30484-7. doi: https://doi.org/10.1016/j.neuropharm.2017.10.016

Arunotayanun W, Dalley JW, Huang X-P, Setola V, Treble R, Iversen L, Roth BL, Gibbons S (2013) An analysis of the synthetic tryptamines AMT and 5-MeO-DALT: emerging 'Novel Psychoactive Drugs'. Bioorg Med Chem Lett 23:3411–3415

Bäckberg M, Beck O, Jönsson K-H, Helander A (2015a) Opioid intoxications involving butyrfentanyl, 4-fluorobutyrfentanyl, and fentanyl from the Swedish STRIDA project. Clin Toxicol 53:609–617

Bäckberg M, Lindeman E, Beck O, Helander A (2015b) Characteristics of analytically confirmed 3-MMC-related intoxications from the Swedish STRIDA project. Clin Toxicol 53:46–53

Baumann MH, Ayestas MA, Dersch CM, Brockington A, Rice KC, Rothman RB (2000) Effects of phentermine and fenfluramine on extracellular dopamine and serotonin in rat nucleus accumbens: therapeutic implications. Synapse 36:102–113

Baumann MH, Partilla JS, Lehner KR (2013a) Psychoactive "bath salts": not so soothing. Eur J Pharmacol 698:1–5

Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF, Holy M, Rothman RB, Goldberg SR, Lupica CR, Sitte HH, Brandt SD, Tella SR, Cozzi NV, Schindler CW (2013b) Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive 'Bath Salts' products. Neuropsychopharmacology 38:552–562

Beck O, Franzen L, Backberg M, Signell P, Helander A (2015) Intoxications involving MDPV in Sweden during 2010-2014: results from the STRIDA project. Clin Toxicol (Phila) 53:865–873

Beck O, Franzen L, Backberg M, Signell P, Helander A (2016) Toxicity evaluation of alpha-pyrrolidinovalerophenone (alpha-PVP): results from intoxication cases within the STRIDA project. Clin Toxicol (Phila) 54:568–575

Blough BE, Landavazo A, Partilla JS, Baumann MH, Decker AM, Page KM, Rothman RB (2014) Hybrid dopamine uptake blocker–serotonin releaser ligands: a new twist on transporter-focused therapeutics. ACS Med Chem Lett 5:623–627

Blundell MS, Dargan PI, Wood DM (2017) The dark cloud of recreational drugs and vaping. QJM 111(3):145–148

Borek HA, Holstege CP (2012) Hyperthermia and multiorgan failure after abuse of "bath salts" containing 3,4-methylenedioxypyrovalerone. Ann Emerg Med 60:103–105

Braden MR, Parrish JC, Naylor JC, Nichols DE (2006) Molecular interaction of serotonin 5-HT2A receptor residues Phe339$^{(6.51)}$ and Phe340$^{(6.52)}$ with superpotent N-benzyl phenethylamine agonists. Mol Pharmacol 70:1956–1964

Brandt SD, Freeman S, Sumnall HR, Measham F, Cole J (2011) Analysis of NRG 'legal highs' in the UK: identification and formation of novel cathinones. Drug Test Anal 3:569–575

Brunt TM, Atkinson AM, Nefau T, Martinez M, Lahaie E, Malzcewski A, Pazitny M, Belackova V, Brandt SD (2017) Online test purchased new psychoactive substances in 5 different European countries: a snapshot study of chemical composition and price. Int J Drug Policy 44:105–114

CDC (2013) Acute kidney injury associated with synthetic cannabinoid use – multiple states, 2012. Morb Mortal Wkly Rep 62:93–98

Clarke S, Dargan P, Jones A (2005) Naloxone in opioid poisoning: walking the tightrope. Emerg Med J 22:612–616

Cole JB, Dunbar JF, Mcintire SA, Regelmann WE, Slusher TM (2015) Butyrfentanyl overdose resulting in diffuse alveolar hemorrhage. Pediatrics 135:e740–e743

Coppola M, Mondola R (2015) AH-7921: a new synthetic opioid of abuse. Drug Alcohol Rev 34:109–110
Domanski K, Kleinschmidt KC, Schulte JM, Fleming S, Frazee C, Menendez A, Tavakoli K (2017) Two cases of intoxication with new synthetic opioid, U-47700. Clin Toxicol (Phila) 55:46–50
Elliott SP, Hernandez Lopez E (2018) A series of deaths involving Carfentanil in the UK and associated post-mortem blood concentrations. J Anal Toxicol. https://doi.org/10.1093/jat/bkx109
EMCDDA (2017) European drug report 2017: trends and developments
Forrester MB (2014) NBOMe designer drug exposures reported to Texas poison centers. J Addict Dis 33:196–201
Gussow L (2016) Toxicology rounds: who said the opioid crisis couldn't get any worse? Emerg Med News 38(1):29–30
Halberstadt AL (2017) Pharmacology and toxicology of N-Benzylphenethylamine ("NBOMe") hallucinogens. Curr Top Behav Neurosci 32:283–311
Helander A, Bäckberg M, Hultén P, Al-Saffar Y, Beck O (2014) Detection of new psychoactive substance use among emergency room patients: results from the Swedish STRIDA project. Forensic Sci Int 243:23–29
Helander A, Bäckberg M, Beck O (2016) Intoxications involving the fentanyl analogs acetylfentanyl, 4-methoxybutyrfentanyl and furanylfentanyl: results from the Swedish STRIDA project. Clin Toxicol 54:324–332
Helander A, Backberg M, Signell P, Beck O (2017) Intoxications involving acrylfentanyl and other novel designer fentanyls – results from the Swedish STRIDA project. Clin Toxicol (Phila) 55:589–599
Hermanns-Clausen M, Müller D, Kithinji J, Angerer V, Franz F, Eyer F, Neurath H, Liebetrau G, Auwärter V (2017) Acute side effects after consumption of the new synthetic cannabinoids AB-CHMINACA and MDMB-CHMICA. Clin Toxicol. https://doi.org/10.1080/15563650.2017.1393082
Heyerdahl F, Hovda KE, Giraudon I, Yates C, Dines AM, Sedefov R, Wood DM, Dargan PI (2014) Current European data collection on emergency department presentations with acute recreational drug toxicity: gaps and national variations. Clin Toxicol 52:1005–1012
Hikin L, Smith PR, Ringland E, Hudson S, Morley SR (2018) Multiple fatalities in the north of England associated with synthetic fentanyl analogue exposure: detection and quantitation a case series from early 2017. Forensic Sci Int 282:179–183
Hill SL, Thomas SH (2011) Clinical toxicology of newer recreational drugs. Clin Toxicol (Phila) 49:705–719
Hill SL, Harbon SC, Coulson J, Cooper GA, Jackson G, Lupton DJ, Vale JA, Thomas SH (2014) Methoxetamine toxicity reported to the National Poisons Information Service: clinical characteristics and patterns of enquiries (including the period of the introduction of the UK's first temporary class drug order). Emerg Med J 31:45–47
Hill SL, Najafi J, Dunn M, Acheampong P, Kamour A, Grundlingh J, Blain PG, Thomas SH (2016) Clinical toxicity following analytically confirmed use of the synthetic cannabinoid receptor agonist MDMB-CHMICA. A report from the identification of novel psycho active substances (IONA) study. Clin Toxicol (Phila) 54:638–643
Hill SL, Dunn M, Cano C, Harnor SJ, Hardcastle IR, Grundlingh J, Dargan PI, Wood DM, Tucker S, Bartram T, Thomas SHL (2018) Human toxicity caused by Indole and Indazole carboxylate synthetic cannabinoid receptor agonists: from horizon scanning to notification. Clin Chem 64:346–354
Hofer KE, Grager B, Müller DM, Rauber-Lüthy C, Kupferschmidt H, Rentsch KM, Ceschi A (2012) Ketamine-like effects after recreational use of Methoxetamine. Ann Emerg Med 60:97–99
Huffman JW, Szklennik PV, Almond A, Bushell K, Selley DE, He H, Cassidy MP, Wiley JL, Martin BR (2005) 1-Pentyl-3-phenylacetylindoles, a new class of cannabimimetic indoles. Bioorg Med Chem Lett 15:4110–4113

James D, Adams RD, Spears R, Cooper G, Lupton DJ, Thompson JP, Thomas SH (2011) Clinical characteristics of mephedrone toxicity reported to the U.K. National Poisons Information Service. Emerg Med J 28:686–689

Jones MJ, Hernandez BS, Janis GC, Stellpflug SJ (2017) A case of U-47700 overdose with laboratory confirmation and metabolite identification. Clin Toxicol 55:55–59

Kamour A, James D, Spears R, Cooper G, Lupton DJ, Eddleston M, Thompson JP, Vale AJ, Thanacoody HK, Hill SL, Thomas SH (2014) Patterns of presentation and clinical toxicity after reported use of alpha methyltryptamine in the United Kingdom. A report from the UK National Poisons Information Service. Clin Toxicol (Phila) 52:192–197

Karass M, Chugh S, Andries G, Mamorska-Dyga A, Nelson JC, Chander PN (2017) Thrombotic microangiopathy associated with synthetic cannabinoid receptor agonists. Stem Cell Investig 4:43

Katselou M, Papoutsis I, Nikolaou P, Spiliopoulou C, Athanaselis S (2015) AH-7921: the list of new psychoactive opioids is expanded. Forensic Toxicol 33:195–201

Miner NB, O'Callaghan JP, Phillips TJ, Janowsky A (2017) The combined effects of 3,4-methylenedioxymethamphetamine (MDMA) and selected substituted methcathinones on measures of neurotoxicity. Neurotoxicol Teratol 61:74–81

Morris H, Wallach J (2014) From PCP to MXE: a comprehensive review of the non-medical use of dissociative drugs. Drug Test Anal 6:614–632

Müller S, Nussbaumer S, Plitzko G, Ludwig R, Weinmann W, Krähenbühl S, Liakoni E (2018) Recreational use of carfentanil – a case report with laboratory confirmation. Clin Toxicol 56:151–152

Nagai F, Nonaka R, Satoh Hisashi Kamimura K (2007) The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. Eur J Pharmacol 559:132–137

Nichols DE, Sassano MF, Halberstadt AL, Klein LM, Brandt SD, Elliott SP, Fiedler WJ (2015) N-Benzyl-5-methoxytryptamines as potent serotonin 5-HT(2) receptor family agonists and comparison with a series of Phenethylamine analogues. ACS Chem Nerosci 6:1165–1175

Omer TA, Doherty C (2011) Posterior reversible encephalopathy syndrome (PRES) complicating the 'legal high' mephedrone. BMJ Case Rep 2011

ONS, OFNS (2017) Statistical bulletin: deaths related to drug poisoning in England and Wales: 2016 registrations

Papaseit E, Pérez-Mañá C, Mateus JA, Pujadas M, Fonseca F, Torrens M, Olesti E, de la Torre R, Farré M (2016) Human pharmacology of mephedrone in comparison with MDMA. Neuropsychopharmacology 41(11):2704–2713

Papaseit E, Molto J, Muga R, Torrens M, De La Torre R, Farre M (2017) Clinical pharmacology of the synthetic cathinone mephedrone. Curr Top Behav Neurosci 32:313–331

Patel N, Ford L, Jones R, Bradberry SM, Vale JA (2017) Poisoning to alpha-pyrrolidinovalerophenone (alpha-PVP), a synthetic cathinone. Clin Toxicol (Phila) 55:159–160

Penders TM, Gestring RE, Vilensky DA (2012) Intoxication delirium following use of synthetic Cathinone derivatives. Am J Drug Alcohol Abuse 38:616–617

Pereira J, Lawlor P, Vigano A, Dorgan M, Bruera E (2001) Equianalgesic dose ratios for opioids: a critical review and proposals for long-term dosing. J Pain Symptom Manage 22:672–687

Rickli A, Luethi D, Reinisch J, Buchy D, Hoener MC, Liechti ME (2015) Receptor interaction profiles of novel N-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). Neuropharmacology 99:546–553

Roth BL, Gibbons S, Arunotayanun W, Huang X-P, Setola V, Treble R, Iversen L (2013) The ketamine analogue methoxetamine and 3- and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor. PLoS One 8:e59334

Rudd RA, Aleshire N, Zibbell JE, Gladden RM (2016) Increases in drug and opioid overdose deaths – United States, 2000-2014. MMWR Morb Mortal Wkly Rep 64:1378–1382

Saha K, Partilla JS, Lehner KR, Seddik A, Stockner T, Holy M, Sandtner W, Ecker GF, Sitte HH, Baumann MH (2015) 'Second-generation' mephedrone analogs, 4-MEC and 4-MePPP, differentially affect monoamine transporter function. Neuropsychopharmacology 40:1321–1331

Samra K, Boon IS, Packer G, Jacob S (2017) Lethal high: acute disseminated encephalomyelitis (ADEM) triggered by toxic effect of synthetic cannabinoid black mamba. BMJ Case Rep 2017. pii: bcr-2016-218431. doi: https://doi.org/10.1136/bcr-2016-218431

Schneir A, Metushi IG, Sloane C, Benaron DJ, Fitzgerald RL (2017) Near death from a novel synthetic opioid labeled U-47700: emergence of a new opioid class. Clin Toxicol (Phila) 55:51–54

Schumann H, Erickson T, Thompson TM, Zautcke JL, Denton JS (2008) Fentanyl epidemic in Chicago, Illinois and surrounding Cook County. Clin Toxicol 46:501–506

Sedefov R, Gallegos A, Mounteney J, Kenny P (2013) Monitoring novel psychoactive substances. A global perspective. In: Dargan PI, Wood DM (eds) Novel psychoactive substances: classification, pharmacology and toxicology. Academic, pp 29–54

Shields JE, Dargan PI, Wood DM, Puchnarewicz M, Davies S, Waring WS (2012) Methoxetamine associated reversible cerebellar toxicity: three cases with analytical confirmation. Clin Toxicol (Phila) 50:438–440

Simmler LD, Liechti ME (2017) Interactions of cathinone NPS with human transporters and receptors in transfected cells. In: Baumann MH, Glennon RA, Wiley JL (eds) Neuropharmacology of new psychoactive substances (NPS): the science behind the headlines. Springer International Publishing, Cham

Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu LH, Huwyler J, Chaboz S, Hoener MC, Liechti ME (2013) Pharmacological characterization of designer cathinones in vitro. Br J Pharmacol 168:458–470

Sivagnanam K, Chaudari D, Lopez P, Sutherland ME, Ramu VK (2013) "Bath salts" induced severe reversible cardiomyopathy. Am J Case Rep 14:288–291

Suzuki J, Dekker MA, Valenti ES, Arbelo Cruz FA, Correa AM, Poklis JL, Poklis A (2015) Toxicities associated with NBOMe ingestion – a novel class of potent hallucinogens: a review of the literature. Psychosomatics 56:129–139

Tait RJ, Caldicott D, Mountain D, Hill SL, Lenton S (2016) A systematic review of adverse events arising from the use of synthetic cannabinoids and their associated treatment. Clin Toxicol (Phila) 54:1–13

Thomas S, Dunn M, Hill SL, Abouchedid R, Dargan P, Wood D, Acheampong P, Kamour A, Tucker S, Grundlingh J, Officer J, Eddleston M (2016) Analytically confirmed exposure to novel psychoactive substances in patients presenting to hospital with severe clinical toxicity in the United Kingdom. Results from the identification of novel psycho active substances (IONA) study (2016). Clin Toxicol 54:713–714

Ukaigwe A, Karmacharya P, Donato A (2014) A gut gone to pot: a case of cannabinoid hyperemesis syndrome due to K2, a synthetic cannabinoid. Case Rep Emerg Med 2014:3

Umebachi R, Aoki H, Sugita M, Taira T, Wakai S, Saito T, Inokuchi S (2016) Clinical characteristics of alpha-pyrrolidinovalerophenone (alpha-PVP) poisoning. Clin Toxicol (Phila) 54(7):563

UNODC (2018) Understanding the synthetic drug market: the NPS factor. Global SMART Update 19

Vallersnes OM, Dines AM, Wood DM, Yates C, Heyerdahl F, Hovda KE, Giraudon I, Dargan PI (2016) Psychosis associated with acute recreational drug toxicity: a European case series. BMC Psychiatry 16:293

Wagmann L, Maurer H (2018) Bioanalytical methods for new psychoactive substances. Handb Exp Pharmacol. doi: https://doi.org/10.1007/164_2017_83

Wikström M, Thelander G, Nyström I, Kronstrand R (2010) Two fatal intoxications with the new designer drug methedrone (4-methoxymethcathinone). J Anal Toxicol 34:594–598

Wiley JL, Lowe JA, Balster RL, Martin BR (1995) Antagonism of the discriminative stimulus effects of delta 9-tetrahydrocannabinol in rats and rhesus monkeys. J Pharmacol Exp Ther 275:1–6

Wiley JL, Marusich JA, Lefever TW, Grabenauer M, Moore KN, Thomas BF (2013) Cannabinoids in disguise: Δ9-tetrahydrocannabinol-like effects of tetramethylcyclopropyl ketone indoles. Neuropharmacology 75:145–154

Wiley JL, Marusich JA, Lefever TW, Antonazzo KR, Wallgren MT, Cortes RA, Patel PR, Grabenauer M, Moore KN, Thomas BF (2015) AB-CHMINACA, AB-PINACA, and FUBIMINA: affinity and potency of novel synthetic cannabinoids in producing Δ9-tetrahydrocannabinol-like effects in mice. J Pharmacol Exp Ther 354:328–339

Winstock AR, Mitcheson LR, Deluca P, Davey Z, Corazza O, Schifano F (2011) Mephedrone, new kid for the chop? Addiction 106:154–161

Winstock A, Lynskey M, Borschmann R, Waldron J (2015) Risk of emergency medical treatment following consumption of cannabis or synthetic cannabinoids in a large global sample. J Psychopharmacol 29:698–703

Wood DM, Davies S, Greene SL, Button J, Holt DW, Ramsey J, Dargan PI (2010) Case series of individuals with analytically confirmed acute mephedrone toxicity. Clin Toxicol (Phila) 48:924–927

Wood DM, Davies S, Puchnarewicz M, Johnston A, Dargan PI (2012) Acute toxicity associated with the recreational use of the ketamine derivative methoxetamine. Eur J Clin Pharmacol 68:853–856

Wood DM, Hill SL, Thomas SH, Dargan PI (2014) Using poisons information service data to assess the acute harms associated with novel psychoactive substances. Drug Test Anal 6:850–860

Wood DM, Sedefov R, Cunningham A, Dargan PI (2015) Prevalence of use and acute toxicity associated with the use of NBOMe drugs. Clin Toxicol (Phila) 53:85–92

Wood DM, Ceronie B, Dargan PI (2016) Healthcare professionals are less confident in managing acute toxicity related to the use of new psychoactive substances (NPS) compared with classical recreational drugs. QJM 109:527–529

Fatal Poisonings Associated with New Psychoactive Substances

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Abstract

This chapter describes how new psychoactive substances (NPS) have been involved in fatal intoxications from 2010 and onwards. It summarizes the circumstances, antemortem symptoms, and adverse effects that have led to death after ingestion of one or more NPS and tabulates concentrations, and postmortem findings from these intoxications.

Consumption of NPS exerts health problems and unknown risks for the users. Data on toxicity of many NPS are scarce or nonexistent and long-term toxicity and risks are still largely unknown. In addition, purity and composition of products containing NPS are often inconsistent or not known, which places users at high risk as evidenced by hospital emergency admissions and deaths.

The most serious threat to drug users are the synthetic opioids that with strong central nervous depressant effects have caused numerous accidental deaths spread over the entire globe. The synthetic cannabinoids seem to be the most unpredictable with no clear toxidrome and unknown or poorly understood mechanisms of toxicity, but with adverse effects pointing toward the cardiovascular system. The toxidromes commonly encountered after ingestion of cathinones and phenethylamines are of sympathomimetic and hallucinogenic character, which includes risk of developing a serotonin syndrome, excited delirium, and life-threatening cardiovascular effects. In comparison to their conventional "parent" drug, i.e., heroin, cannabis, and amphetamine, most NPS appear to exhibit more severe adverse effects. The deaths attributed to NPS have dramatically increased in the last years. In our opinion, this is because of the shift from synthetic cannabinoids and cathinones to the even more toxic and dangerously potent fentanyl analogues.

Keywords

Cathinones · Death · Designer drugs · Fatal intoxications · New psychoactive substances · Opioids · Phenethylamines · Postmortem concentrations · Synthetic cannabinoids

Acronyms of the Discussed New Psychoactive Substances (NPS)

Name used in this chapter	Chemical name and pseudonyms
Synthetic opioids	
Acetylfentanyl	*N*-[1-(2-phenylethyl)-4-piperidyl]-*N*-phenylacetamide
Acrylfentanyl	*N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]prop-2-enamide (aka. acryloylfentanyl)
AH-7921	3,4-Dichloro-*N*-([1-(dimethylamino)cyclohexyl]methyl) benzamide
Beta-hydroxythiofentanyl	*N*-[1-(2-hydroxy-2-thiophen-2-ylethyl)piperidin-4-yl]-*N*-phenylpropanamide
Butyrylfentanyl	*N*-(1-phenethylpiperidin-4-yl)-*N*-phenylbutyramide (aka. butyrfentanyl)

(continued)

Carfentanil	Methyl 1-(2-phenylethyl)-4-[phenyl(propanoyl)amino] piperidine-4-carboxylate
4-Chloroisobutyrfentanyl	*N*-(4-chlorophenyl)-*N*-(1-phenethylpiperidin-4-yl) isobutyramide
Despropionylfentanyl	*N*-phenyl-1-(2-phenylethyl)piperidin-4-amine (aka. 4-ANPP)
4-Fluoroisobutyrfentanyl	*N*-(4-fluorophenyl)-*N*-(1-phenethylpiperidin-4-yl) isobutyramide
Isobutyrylfentanyl	2-Methyl-*N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl] propanamide
Furanylfentanyl	*N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]furan-2-carboxamide (aka. Fu-F)
Loperamide	4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide
3-Methylfentanyl	*N*-[3-Methyl-1-(2-phenylethyl)piperidin-4-yl]-*N*-phenylpropanamide
MT-45	1-Cyclohexyl-4-(1,2-diphenylethyl)piperazine
Ocfentanil	*N*-(2-fluorophenyl)-2-methoxy-*N*-[1-(2-phenylethyl)piperidin-4-yl]acetamide
para-Fluorobutyrylfentanyl	*N*-(4-fluorophenyl)-*N*-[1-(2-phenylethyl)piperidin-4-yl] butanamide
para-Fluoroisobutyrylfentanyl	*N*-(4-fluorophenyl)-*N*-(1-phenethylpiperidin-4-yl) isobutyramide (aka. 4-fluoroisobutyrylfentanyl, 4F-iBF, 4-FIBF, and pFIBF)
para-fluorofentanyl	*N*-(4-fluorophenyl)-*N*-[1-(2-phenylethyl)piperidin-4-yl] propanamide (aka. 4-Fluorofentanyl)
Tetrahydrofuranfentanyl	*N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]oxolane-2-carboxamide (aka. THF-F)
U-47700	3,4-Dichloro-N-[(1*R*,2*R*)-2-(dimethylamino)cyclohexyl]-*N*-methylbenzamide
Synthetic cannabinoids	
5F-ADB	Methyl 2-([1-(5-fluoropentyl)-1*H*-indazole-3-carbonyl] amino)-3,3-dimethylbutanoate (aka. 5F-MDMB-PINACA)
5F-AMB	Methyl *N*-[1-(5-fluoropentyl)-1*H*-indazole-3-yl] carbonylvalinate
5F-AKB-48	1-(5-Fluoropentyl)-*N*-(tricyclo[3.3.1.1^{3,7}]dec-1-yl)-1H-indazole-3-carboxamide
5F-PB-22	Quinolin-8-yl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate
AB-CHMINACA	*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide
AB-FUBINACA	*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide
AB-PINACA	*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1*H*-indazole-3-carboxamide
ADB-CHMINACA	*N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide
ADB-FUBINACA	*N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-[(4-fluorophenyl)methyl]indazole-3-carboxamide

(continued)

ADB-PINACA	*N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentylindazole-3-carboxamide
AKB-48	1-Pentyl-*N*-tricyclo[3.3.1.1^{3,7}]dec-1-yl-1H-indazole-3-carboxamide (aka. APINACA)
AM-1220	[1-[(1-Methylpiperidin-2-yl)methyl]indol-3-yl]-naphthalen-1-ylmethanone
AM-2201	[1-(5-Fluoropentyl)-1H-indol-3-yl](naphthalen-1-yl) methanone
AM-2232	5-[3-(Naphthalene-1-carbonyl)indol-1-yl]pentanenitrile
AM-694	[1-(5-Fluoropentyl)indol-3-yl]-(2-iodophenyl)methanone
Cumyl-4CN-BINACA	1-(4-Cyanobutyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indazole-3-carboxamide
FUB-PB-22	Quinolin-8-yl 1-[(4-fluorophenyl)methyl]indole-3-carboxylate
JWH-018	Naphthalene-1-yl(1-pentyl-1*H*-indol-3-yl)methanone
JWH-073	(1-Butyl-1*H*-indol-3-yl)(naphthalen-1-yl)methanone
JWH-122	(4-Methylnaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone
JWH-210	(4-Ethylnaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone
MAM-2201	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](4-methylnaphthalen-1-yl) methanone
MDMB-CHMICA	Methyl 2-[[1-(cyclohexylmethyl)-1*H*-indole-3-carbonyl] amino]-3,3-dimethylbutanoate (aka. MMB-CHMINACA)
NNEI	1-(5-Fluoropentyl)-*N*-(tricyclo[3.3.1.1^{3,7}]dec-1-yl)-1*H*-indole-3-carboxamide
STS-135	1-(5-Fluoropentyl)-*N*-(tricyclo[3.3.1.1^{3,7}]dec-1-yl)-1*H*-indole-3-carboxamide
THJ-2201	[1-(5-Fluoropentyl)-1*H*-indazol-3-yl](naphthalen-1-yl) methanone
UR-144	(1-Pentyl-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl) methanone
XLR-11	[1-(5-Fluoropentyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone
Synthetic cathinones and phenethylamines	
25B-NBOMe	2-(4-Bromo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl) methyl]ethanamine
25C-NBOMe	2-(4-Chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl) methyl]ethanamine
25H-NBOMe	2-(2,5-Dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethanamine
25I-NBOMe	2-(4-Iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl) methyl]ethanamine
3,4-DMMC	1-(3,4-Dimethylphenyl)-2-(methylamino)propan-1-one (aka. 3,4-dimethylmethcathinone)
3-MMC	2-(Methylamino)-1-(3-methylphenyl)-1-propanone (aka. 3-methylmethcathinone)
5-APB	1-(1-Benzofuran-5-yl)propan-2-amine
5-IT	5-(2-Aminopropyl)indole
Alpha-PVP	1-Phenyl-2-(pyrrolidin-1-yl)pentan-1-one (aka. α-PVP, α-pyrrolidinovalerophenone)

(continued)

Butylone	1-(1,3-Benzodioxol-5-yl)-2-(methylamino)butan-1-one
DOI	2,5-Dimethoxy-4-iodoamphetamine
MDPV	1-(1,3-Benzodioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one
Mephedrone	2-Methylamino-1-(4-methylphenyl)propan-1-one (aka. 4-methylmethcathinone)
Methedrone	1-(4-Methoxyphenyl)-2-(methylamino)propan-1-one (aka. 4-Methoxy-N-methylcathinone)
Methylone	2-Methylamino-1-(3,4-methylenedioxyphenyl)propan-1-one
N-ethylnorpentylone	1-(2H-1,3-Benzodioxol-5-yl)-2-(ethylamino)pentan-1-one (aka. N-ethylpentylone)
Pentedrone	(±)-2-(Methylamino)-1-phenylpentan-1-one (aka. α-methylaminovalerophenone)
PMA	1-(4-Methoxyphenyl)propan-2-amine (aka. p-Methoxyamphetamine)
PMMA	[1-(4-Methoxyphenyl)propane-2-yl](methyl)azane]] (aka. 4-methoxymethylamphetamine, 4-MMA)
Miscellaneous	
BZP	1-Benzylpiperazine
Diphenidine	1-(1,2-Diphenylethyl)piperidine
Methoxetamine	2-(3-Methoxyphenyl)-2-(ethylamino)cyclohexanone (aka. MXE)
Methoxydiphenidine	1-[1-(2-Methoxyphenyl)-2-phenylethyl]piperidine (aka. methoxphenidine, MXP)
TFMPP	1-(3-Trifluoromethylphenyl)piperazine

1 Introduction

The administration of drugs and substances used for recreational purposes has caused both accidental and non-accidental deaths throughout history. With the introduction of new psychoactive substances (NPS) during the last decade, a new challenge has arisen for the interpretation of postmortem drug concentrations and determination of cause of death due to substances with unknown potency and possible off-target toxicities (Elliott et al. 2018). NPS have often been linked to health problems and unknown risks of harm to users. In general, the development of clinical features associated with NPS use might range from CNS toxicity resulting in seizures, aggression, acute psychosis as well as cardiotoxicity and liver toxicity resulting in arrhythmias and system failures. Severe NPS intoxications may have a fatal outcome even if the individual has been hospitalized, but most deaths occur when the decedent is alone without the support of intensive care. Safety data on toxicity and lethality of many NPS are scarce or nonexistent and long-term toxicity and risks are still largely unknown. In addition, purity and composition of products containing NPS are often inconsistent or not known, which places users at high risk as evidenced by hospital emergency admissions and deaths, sometimes as a result of

polysubstance use. Dangerously high levels of toxins or drugs in the body often lead to a syndrome or set of symptoms specific for the mechanism of the individual drug/toxin. This set of symptoms is often called a toxidrome, a term first used by Mofenson and Greensher (1970). Common examples are the cholinergic, the anticholinergic, the sympathomimetic, the hallucinogenic, the sedative/hypnotic, and opioid toxidrome. Conventional drugs of abuse such as heroin, amphetamine, cocaine, and benzodiazepines are known to produce a certain toxidrome, whereas NPS are less well understood or when taken in combination present with various, mixed symptoms. In this chapter, we summarize the circumstances, antemortem symptoms, and adverse effects that have led to death after ingestion of one or more NPS and tabulate concentrations, and postmortem findings from these intoxications.

2 Methods

Case studies published between 2010 and 2017 were identified through PubMed searches of drug groups or individual NPS names in combination with either of the words "fatal," "death," "postmortem," or "intoxication." In addition to journal articles, we also used risk assessment reports from EMCDDA and critical review reports from the World Health Organization's Expert Committee on Drug Dependence (ECDD) to find references to fatal intoxications. Articles were also searched for adequate references. This was combined with the literature describing the toxicology of the drugs as well as public reports of prevalence from European, American, and international agencies. The summary was limited to cases in which the drug was analytically confirmed. Some cases in which the cause of death was induced by the drug have been included as well as reference values in other populations when supplied by the references. When grouping cases from several papers, concentrations in mass units (g) have been converted to volume (mL).

3 Synthetic Opioids

Synthetic opioids are responsible for the majority of fatal intoxications worldwide. Along with the rise of heroin abuse in the USA, increasing steadily since 2011, a dramatic increase in the abuse of the prescription opioid fentanyl has been registered from 2013 onward (UNODC 2017a).

Although only adding up to 4% of all the NPS appearing on the market on a monthly basis (2016), opioid NPS (or research opioids) represent a constant and increasingly popular danger. Since 2012, the number of newly reported synthetic opioids is steadily increasing, starting from three compounds in 2012 (two fentanyl analogs and one other compound) up to ten new substances in 2016 (nine fentanyl analogs and one other compound) (UNODC 2017b).

The opioid toxidrome is characteristic for all opioid receptor-targeting molecules, and clinical symptoms are commonly found in the majority of autopsy investigations involving synthetic opioid overdose cases. Among the most common and lethal

symptoms was respiratory depression, marked by pulmonary edema, cerebral edema, and distended bladder, along with other possibly substance-specific symptoms, e.g., cardiotoxicity induced by loperamide, or the presence of cardiomegaly in carfentanil cases (Eiden et al. 2012; White and Irvine 1999; Winklhofer et al. 2014). Other complications of the opioid toxidrome might include tachycardia as a response to hypoxia or hypercardia, peripheral vasodilatation with hypothermia and consequent hypotension (Khademi et al. 2016). Moreover, loperamide is normally prevented from entering into the central nervous system (CNS) by its high affinity with the P-glycoprotein (P-gp) efflux transporter on the blood–brain barrier (loperamide efflux:influx ratio is 10 in vitro and 10.4 in situ); any P-gp inhibitor drugs present in the system would highly increase the CNS distribution of loperamide – and to various degree of opioids in general – thus increasing the risk of fatal complications (Mercer and Coop 2011).

3.1 MT-45

The piperazine derivative MT-45 represents a signifcanty different structure from that of classic opiates (e.g., morphine). However, the ability to activate the opioid receptors is retained, and it mediates analgesic activity comparable to morphine. Interestingly, the highest affinity of MT-45 is for the δ- and κ-opioid receptor (DOR and KOR) ($IC_{50} = 156$ and 176 nM, respectively), rather than for μ-opioid receptor (MOR) ($IC_{50} = 644$ nM) unlike morphine (IC_{50} morphine at MOR: 4.6 nM, DOR: 78.6 nM, and KOR 242 nM) (ECDD 2015). Analgesic ED_{50} values for MT-45 in rats were reported to be 6.62 mg/kg (against thermal pain, more effective than morphine) and 0.73 mg/kg (against mechanical pain, less effective than morphine) (Nakamura and Shimizu 1976). Since 2016, MT-45 is listed in Schedule 1 of the Single Convention on Narcotic Drugs 1961 as amended by the 1972 Protocol (UNODC 2017b). An MT-45 fatal intoxication case occurred in Germany, as documented by Fels et al. (2017), in which the high concentration of MT-45 in femoral blood and the lack of other plausible causes determined intoxication by MT-45 as the main cause of death (Table 1).

3.2 AH-7921

The compound AH-7921 reportedly presents 80% of morphine's agonist activity, nonetheless showing – at least in rodents – to be more effective at nociceptive reduction than the same dose of morphine (e.g., phenylquinone test, ED_{50} morphine: 1.1 mg/kg, ED_{50} AH-7921: 0.85), while at the same time inducing more dangerous respiratory depression and reduced pulse rate (Brittain et al. 1973; Hayes and Tyers 1983). Its nociceptive activity is exerted through MOR activation and possibly also through KOR (ECDD 2014). Since 2015, AH-7921 is listed in Schedule 1 of the Single Convention on Narcotic Drugs 1961 as amended by the 1972 Protocol (UNODC 2017b). Thirteen cases of fatal AH-7921 intoxication have been published

Table 1 Summary of the case report data of synthetic opioid poisonings

Substance	Distribution	CoD	N	C range PM blood (ng/mL)	Antemortem and postmortem findings	References
Acetylfentanyl	14 cases. 12 cases in the USA[a] and two in Japan	Intoxication	10	192–2,000	Pulmonary edema, diffuse cerebral edema, and congestion of lungs and other organs	Cunningham et al. (2016); Fort et al. (2016); McIntyre et al. (2015b), (2016); Poklis et al. (2015), (2016); Takase et al. (2016); Yonemitsu et al. (2016)
		Mixed intoxication	4	6–38		
Acrylfentanyl	40 cases in Sweden[b] and three cases in the USA[f]	Intoxication	2	0.01–0.02	Pulmonary edema, brain edema, and congested lungs	Guerrieri et al. (2017b), Butler et al. (2017)
		Mixed intoxication	41	0.01–5.3		
Butyrylfentanyl	Three cases in the USA[a]	Intoxication	1	58	Myocardial hypertrophy and pulmonary edema	McIntyre et al. (2016); (Poklis et al. 2016)
		Mixed intoxication	2	3.7–99		
Carfentanil	Two cases in the USA[c]	Intoxication	1	0.12 (heart blood)	Left ventricular hypertrophy	Swanson et al. (2017)
		Mixed intoxication	1	1.3 (heart blood)		
Furanylfentanyl	16 cases. Seven cases in Sweden[b] and 11 in the USA[c–e]	Intoxication	6	0.41–76	Pulmonary edema and diffuse cerebral edema	Guerrieri et al. (2017a); Mohr et al. (2016); Swanson et al. (2017) Butler et al. (2017); Martucci et al. (2018)
		Mixed intoxication	11	0.36–12.9		
Ortho-fluorofentanyl	One case in Norway	Mixed intoxication	1	2.4	ND	Helland et al. (2017)
Ocfentanil	One case in Belgium	Intoxication	1	15.3	ND	Coopman et al. (2016b)
Tetrahydro-furanylfentanyl	One case in the USA[f]	Mixed intoxication	1	339	Pulmonary congestion and edema, and cerebral edema	Krotulski et al. 2017

Loperamide	22 cases in the USA (15)	Intoxication	18 (7)	340–890	Respiratory depression, dysrhythmias, central nervous system depression, and cardiotoxicity	Bishop-Freeman et al. (2016); Eggleston et al. (2017); Lasoff et al. (2017); Vakkalanka et al. (2017)
		Mixed intoxication	4 (8)	140–500, 77 in heart blood		
AH-7921	13 cases. Two in Norway, nine in Sweden[b], one in the USA, and one in Germany	Intoxication	4	84–859	Pulmonary and cerebral edema, and internal organ congestion	Fels et al. (2017); Karinen et al. (2014); Kronstrand et al. (2014); Vorce et al. (2014)
		Mixed intoxication	9	318–9,100		
U-47700	23 cases. One in UK, two in Germany, one in Belgium, and 19 in the USA[d]	Intoxication	15	40–487	Pulmonary edema and congestion, and enlarged heart	Coopman et al. (2016a); Dziadosz et al. (2017); Ellefsen et al. (2017); Elliott et al. (2016); Fels et al. (2017); McIntyre et al. (2017); Mohr et al. (2016); Rohrig et al. (2017)
		Mixed intoxication	8	13.8–490		
U-49900	One case in the USA[f]	Mixed intoxication	1	1.5	Pulmonary congestion and edema, and cerebral edema	Krotulski et al. (2017)
MT-45	One case in Germany	Mixed intoxication	1	2,900	Pulmonary edema, and cerebral edema	Fels et al. (2017)

ND not detected, *CoD* cause of death
[a]Two cases presented both acetylfentanyl and butyrylfentanyl
[b]Swedish cases converted from ng/g (1.06 kg/m^3 blood density)
[c]One case presented both furanylfentanyl and carfentanil
[d]Five cases presented both U-47700 and furanylfentanyl
[e]One case presented both acrylfentanyl and furanylfentanyl
[f]One case presented both U-49900 and tetrahydro-furanylfentanyl

by groups from Sweden (nine cases), Norway (two cases), Germany (one case), and the USA (one case); as per the opioid toxidrome, the deceased commonly presented pulmonary and cerebral edema. In four cases, AH-7921 was determined to be the cause of death alone, and in nine cases combinations with other drugs of abuse were noted (Table 1).

3.3 U-47700

The AH-7921 structural isomer U-47700 was originally synthesized in the 1970s and showed the highest affinity toward the MOR (Ki = 0.91 nM), followed by the KOR (Ki = 110 nM) and DOR (Ki = 480 nM). In vitro assays results ($[^{35}S]GTP\gamma S$) revealed EC_{50} values of 140 nM (MOR), 201 nM (KOR), and 4,540 nM (DOR). The functional activity values for morphine obtained from the same assay were 31, 83, and 870 nM. Its nociceptive potency tested using the tail-flick assay had an ED_{50} of 0.2 mg/kg (7.5 more potent than morphine) (ECDD 2016; Szmuszkovicz and Von Voigtlander 1982). U-47700 has no registered medical use and, since 2017, is listed in Schedule 1 of the Single Convention on Narcotic Drugs 1961 as amended by the 1972 Protocol (UNODC 2017b). Twenty-three cases of fatal U-47700 intoxications have been published in the literature, one case from UK, two from Germany, one from Belgium, and 19 from the USA. The compound has been identified to be the cause of death alone (15 cases) or in combination with other drugs of abuse (eight cases), inducing symptoms compatible with the opioid toxidrome: common findings were pulmonary and cerebral edema, along with a less common enlargement of the heart. See also Table 1.

3.4 Fentanyl Analogs

Fentanyl is a powerful synthetic opioid with a 80–100-fold higher potency than morphine commonly used as pain medication; indeed, by monitoring pain levels in cancer patients switching between fentanyl and morphine, the relative/equivalent analgesic potency of fentanyl was calculated being of 68:1 (15–100:1), 84:1 (65–112.5:1), and 66:1 (see Pereira et al. 2001 for a compiled collection of individual studies). After fentanyl was placed under international control, research carried out between 1960s and 1990s expanded the fentanyl class to include numerous analogs and derivatives. A few eventually were approved for medical use in humans, others in animals, and many more were never developed further. In recent years, drug manufacturers and traffickers rediscovered many of these compounds as well as synthesized new analogs and introduced them to the recreational drug market (UNODC 2017b). As previously reported, fentanyl analogs constitute the vast majority of opioid NPS appearing yearly on the market. The following has no intention to be an exhaustive collection of all cases related to fentanyl analogs. Due to the vast number of fatal cases and complex polydrug toxicological profiles, which often complicate the consolidation of data, it is beyond the scope of this text to

enumerate every single intoxication case. Moreover, compiled world reports with aggregate data on synthetic opioids rarely discriminate between single specific compounds. Nonetheless, at a local level, it is possible to find breakdowns of the aggregate intoxication data, which could potentially be a general indication of the global trend. According to Daniulaityte et al. (2017), 281 cases of lethal intoxication were reported (181 males and 100 females) in Ohio (USA) between January and February 2017: of these, 90% returned positive results for fentanyl, whereas 55.9% of cases showed the presence of norfentanyl (the main fentanyl metabolite), 48.4% of acrylfentanyl, 42% of despropionylfentanyl (metabolite of fentanyl and several fentanyl analogs as well as drug in its own right), 0.4% of despropionyl *para*-fluorofentanyl, 31% of furanylfentanyl, 0.7% of furanyl-norfentanyl, 7.5% of carfentanil, 1.4% of acetylfentanyl, 1.4% of butyryl/isobutyrylfentanyl, 0.7% of butyryl-norfentanyl, 1.1% of fluorobutyryl/fluoroisobutyrylfentanyl, and 0.7% of U-47700. Such a complex distribution profile is likely to be a consequence, at least in part, of the poor quality of products circulating on the recreational drugs market, often resulting in mislabeled, laced, or adulterated products (Mounteney et al. 2015). A similar complex pattern was found in the report of Shoff et al. (2017): 500 fatal intoxications were selected based on case history among the cases reported over the period of 2015 and 2016 in Miami-Dade, Florida (USA). Of these, 375 cases were positive for fentanyl and/or fentanyl analogs. Specifically, 176 cases were positive for six different fentanyl analogs and U-47700: beta-hydroxythiofentanyl (nine cases), acetylfentanyl (13 cases), carfentanil (134 cases), furanylfentanyl (37 cases), *para*-fluoroisobutyrylfentanyl (26 cases), butyrylfentanyl (three cases), and U-47700 (four cases). As per the previously mentioned report, males constituted the majority of the deceased (82%) (Shoff et al. 2017). A broader collection of data can be found in the report published by O'Donnel et al. (2017). The document collects the total opioid overdose deaths from ten USA states in the months between July and December 2016 (Maine, Massachusetts, New Hampshire, New Mexico, Ohio, West Virginia, Wisconsin Missouri, Oklahoma, and Rhode Island). The total number of deaths identified was 5,152, of which 720 showed a presence of at least one fentanyl analog or of U-47700; specifically, there have been 389 carfentanil cases, 182 furanylfentanyl cases, 147 acetylfentanyl cases, 40 U-47700 cases, and 74 cases involving other analogs such as 3-methylfentanyl, acrylfentanyl, butyrylfentanyl, *para*-fluorofentanyl (4-fluorofentanyl), *para*-fluorobutyrylfentanyl (4-fluorobutyrylfentanyl), and *para*-fluoroisobutyrylfentanyl (4-fluoroisobutyrylfentanyl). Again, the report shows the great majority (72.2%) of users to be males (O'Donnell et al. 2017) (Table 1).

3.5 Individual Compounds Case Reports

For a more granular analysis on the specific characteristics of lethal intoxications associated with synthetic opioids, it is possible to sieve through the literature to find individual case reports that feature popular fentanyl analogs and other synthetic

opioids, describing autopsy findings and toxicological profiles. Representative compounds and their respective case report data are compiled in Table 1.

3.6 Naloxone

Although this text aims at discussing cases of fatal intoxication by synthetic opioids, it is also appropriate to mention the role of naloxone in reversing the opioid toxidrome.

Naloxone is a nonspecific competitive opioid receptor antagonist, and commonly used by health professionals to counteract respiratory depression caused by opiates and more frequently by synthetic opioids. Naloxone administration could be intravenous, intramuscular, subcutaneous, or intranasal, and it rapidly distributes throughout the body. Critically, the effect of naloxone may not outlast the respiratory depression caused by the opioid, and therefore multiple doses or parallel supportive treatments must be administered (DailyMed 2014).

Indeed, timely treatment with therapeutic doses of naloxone (singles or continuous until recovery) bears a high rate of success in preventing lethal outcomes of opioid intoxication. For instance, in Sweden, Helander et al. (2017) reported that 11 patients were treated for intoxication by acrylfentanyl (eight cases), acrylfentanyl and 4-chloroisobutyrfentanyl (one case), 4-fluoro-isobutyrylfentanyl (one case), and tetrahydrofuranfentanyl (one case); ten of them received single or continuous administrations of 0.1–0.4 mg naloxone, and nine of them fully recovered (Helander et al. 2017). Similarly, in California (USA), a 41-year-old woman with a polydrug profile containing, among others, U-47700 and fentanyl, was treated with 0.4 mg naloxone intravenously and completely recovered in 4 h (Armenian et al. 2017). Lastly, in Florida (USA), two 24- and 29-year-old males passed out while driving; toxicological screening identified fentanyl, acetylfentanyl, and carfentanil in the first case, and acetylmorphine, morphine, and carfentanil in the second; naloxone treatment successfully reverted the intoxication effects (Tiscione and Shanks 2016).

4 Synthetic Cannabinoid Receptor Agonists

Compared to other NPS classes, in particular the class of synthetic opioids, fatal intoxications with synthetic cannabinoid receptor agonists (SCRAs) are rather the exception than the rule. In many intoxication cases, major complications are severe and include cardiovascular events, acute kidney injury, seizures, hyperemesis, and psychiatric presentations, but often people can be treated symptomatically and then discharged from hospital fully recovered (Tait et al. 2016). However, in some cases, more serious effects such as stroke, myocardial infarction, rhabdomyolysis, liver and kidney failure, and psychosis have been observed and have eventually led directly or indirectly to a fatal outcome. It remains unclear which factors contribute to the individual outcome, but certain preexisting conditions (for instance, an untreated cardiac disease), metabolic particularities, or a developed tolerance might play a role.

Death caused by intoxication with a cannabimimetic agent is a new phenomenon that has only appeared after potent SCRAs were introduced in the NPS market. Fatalities, in which natural cannabis was ruled as a direct cause of death, are extremely rare, as none of the major natural cannabinoids is considered fatal per se. Given that Cannabis is the most widely consumed illicit drug in the world with an annual prevalence of >150 million people (UNODC 2017a), the number of case reports on fatalities, most of them describing cardiovascular adverse effects, is vanishingly small (Bachs and Morland 2001; Dines et al. 2015; Hartung et al. 2014). In contrast to other classes of psychoactive drugs, there is no established classical toxidrome associated with the use of cannabimimetics that would account for all the effects typically seen with excessive stimulation of central and peripheral cannabinoid receptors. Table 2 lists 54 mono- and 13 mixed intoxication cases, in which at least one synthetic cannabinoid was present. The table is based on published scientific papers only and does not include the numerous fatalities that have never been described in a case report, or that went unreported due to lack of toxicological testing. Although the current compilation of cases is small and does not allow for drawing any general conclusions on the nature of a hypothetical "cannabimimetic toxidrome," it provides an impression of the variety of circumstances, compounds involved, and different autopsy findings. The typical fatal case scenario is as follows: the victim is young and male (which is consistent with the profile of a typical NPS consumer) and found dead by friends or family. Fewer cases present as a relatively sudden episode of medical problems, such as trouble breathing, chest pain, unconsciousness, and nausea, following consumption of an SCRA with or in the company of others. Only a minority of fatal cases involves a longer stay at the hospital with progressive deterioration of the patient's condition despite medical treatment. The compounds involved in fatal cases originate from a variety of chemical classes (Fig. 1). Different tails (pentyl, pentenyl, fluoropentyl, cyclohexyl, fluorobenzyl, butyl, and cyanobutyl) as well as different cores (indole and indazole), linkers (carbonyl, ester, and carboxamide), and linked groups (naphthyl, quinoline, adamantyl, tetramethylcyclopropyl, valinamide/valinate, and cumyl) are frequently observed among the culprits. With the current set of cases, it is impossible to determine whether certain structural elements lead to higher toxicity. In addition, the data suffer from many more limitations: prevalence data on individual SCRAs are scarce, analytical methods are largely inconsistent and have significant gaps, and case reports are published rather on a sporadic than on a systematic basis.

As there is no knowledge on a possible physiological mechanism that leads to toxicity, interpretation of cases that involve an SCRA is complicated. The difficulties are illustrated by the variety of determined causes of death, which reflects the different levels of certainty and direct causation. Causes of death range from "intoxication by a synthetic cannabinoid" to "suffocation/hypothermia/suicide/exsanguination/liver failure/sudden death/acute coronary thrombosis, etc. in the presence of a synthetic cannabinoid" to "undetermined, but a contribution of the synthetic cannabinoid found cannot be excluded." To further complicate matters, the most prominent autopsy findings were relatively nonspecific (pulmonary edema, cerebral edema, and congestion of inner organs) or nothing remarkable was found at

Table 2 Summary of the case report data of poisonings with synthetic cannabinoid receptor agonists (SCRAs)

Substance	CoD	N	C blood (ng/mL)	Antemortem and postmortem findings	References
Monointoxications					
JWH-018 (and JWH-073)	Not stated	1	JWH-018: 199 (cardiac)	PM: enlarged heart	Shanks et al. (2012)[a]
	Not stated	1	JWH-018: 19.6 and JWH-073: 68.3	–	
	Suicide by exsanguination after K2 consumption	1	JWH-018: 83.3 (cardiac)	–	
	Not stated	1	JWH-018 confirmed, no quantitative value available		Trecki et al. (2015)
AM2201	Self-inflicted stabbing due to psychiatric episode after AM2201 consumption	1	AM-2201: 12 OH-AM2201: 2.47 5-OH-Pentyl JWH-018: 123 and JWH-018 pentanoic acid: 50.8	PM: blunt-force injuries, large stab wound to the right side of the neck	Patton et al. (2013)
	Not stated	2	AM-2201 confirmed		Trecki et al. (2015)
MAM-2201	Intoxication	1	MAM-2201: 12.4 (PM interval ca. 4 days)	PM: no external injuries or endogenous diseases	Saito et al. (2013)
JWH-210	Hypothermia in combination w/intoxication of psychotropic substances	1	JWH-210: 12 ng/g	PM: lung edema	Kronstrand et al. (2013a)
5F-PB-22	Intoxication	1	5F-PB-22: 1.1	AM: boy began “gasping for air and fell to the ground”	Behonick et al. (2014)
	Fulminant liver failure	1	Three serum samples AM: 5F-PB-22 not tested – not tested – 1.3; THC-COOH: 246 – 176 – ND	AM: liver and kidney injury, coagulopathy, respiratory failure, hypoxemia, anion gap metabolic and lactic acidosis,	

				and brief episode of cardiac arrest	
	Sudden death in combination w/ 5F-PB-22 use	1	5F-PB-22: 1.5 (iliac)	AM: unresponsive, not breathing, pulseless, and cool to the touch	
				PM: bilateral pulmonary vasocongestion and congestion in the abdominal organs	
	Intoxication	1	5F-PB-22: 1.5	PM: bilateral pulmonary edema, necrotizing granulomatous inflammation w/ histoplasma microorganism, and congestion of viscera	
	Not stated	2	5F-PB-22 confirmed		Trecki et al. (2015)
	Suffocation in the presence of 5F-PB-22 and alcohol	1	5F-PB-22: 0.37 (femoral)	PM: acute oxygen deficiency, cerebral and pulmonary edema, and blood congestion of inner organs	Angerer et al. (2017)
NNEI	Acute circulatory disturbance caused by NNEI intoxication	1	NNEI: 0.64 (left atrium)	PM: arteriolar wall hypertrophy, interstitial fibrosis in the heart, marked pulmonary congestion and edema, lymphocytic infiltrations in the liver, and spleen congestion	Sasaki et al. (2015)
XLR-11	Intoxication	1	XLR-11: 1.4	–	Shanks et al. (2015b)
	Undetermined, w/ significant findings of XLR-11 toxicity	1	XLR-11: 0.6	AM: chest pain, nausea, and agitation	

(continued)

Table 2 (continued)

Substance	CoD	N	C blood (ng/mL)	Antemortem and postmortem findings	References
				PM: pulmonary edema and congestion, and acute visceral congestion	
5F-AMB	Intoxication	1	5F-AMB: 0.3	AM: cool to the touch	Shanks and Behonick (2016)
				PM: no remarkable findings	
5F-ADB	Not stated	1	5F-ADB: 0.19 (PM interval ca. 2 days)	PM: no remarkable findings	Kusano et al. (2017)
	Acute circulatory failure after drug inhalation in all four cases	1	5F-ADB: 0.24/0.45 (cardiac right/left)	PM: no remarkable findings	Usui et al. (2017)
		1	5F-ADB: 1.35 (cardiac)	PM: ischemic cardiac disease	
		1	5F-ADB: 0.14/0.11 (cardiac right/left)	PM: no remarkable findings	
		1	5F-ADB: 1.92 (cardiac)	PM: no remarkable findings	
	Not stated	1	5F-ADB: 0.38 in urine	PM: acute oxygen deficiency, cerebral edema, congestion of internal organs, myocardial cells death, and aspiration of stomach content	Angerer et al. (2017)
AB-PINACA	Death associated w/AB-PINACA and ethanol intoxication	1	AB-PINACA: 32.8	PM: pulmonary edema and visceral congestion	Shanks et al. (2014)
	Death associated w/AB-PINACA intoxication	1	AB-PINACA: 12.2 (iliac)	PM: severe pulmonary edema and congestion, and hepatosplenic and bilateral renal vasocongestion	
MDMB-CHMICA	Multiple organ failure	1		AM: fall on the floor moments after smoking, vomiting,	Adamowicz (2016)

			MDMB-CHMICA: 5.6 (AM blood), traces in PM blood	unconsciousness; 30 min later no pulse, GCS 3; and death after 4 days after two cardiac arrests	
				PM: respiratory, circulatory, heart, kidney, and liver failure, and hypoxic-ischemic CNS damage	
	Not stated	1	MDMB-CHMICA: 1.4 (serum, 2 h after collapse)	AM: cardiac arrest 15 min after smoking, asystole, and brain hypoxia	Westin et al. (2016)
ADB-CHMINACA	Not stated	Several cases	ADB-CHMINACA confirmed. Poisoning in Mississippi and Michigan		Kasper et al. (2015)
	Not stated	1	ADB-CHMINACA confirmed		Katz et al. (2016)
	Not stated	2	ADB-CHMINACA confirmed		Trecki et al. (2015)
AB-CHMINACA	Acute cardiorespiratory failure	1	AB-CHMINACA: 1.5	AM: loss of balance and fall to the floor after two puffs, nose bleeding, and slurred speech; after 1 h unconsciousness, weak pulse, and shallow breathing	Gieron and Adamowicz (2016)
				PM: congestion of internal organs and pulmonary edema	
	Intoxication w/AB-CHMINACA in the presence of ethanol	1	AB-CHMINACA: 4.1 (femoral)	PM: cerebral and pulmonary edema	Angerer et al. (2017)
	Intoxication w/AB-CHMINACA	1	AB-CHMINACA: 7.6; trace amounts of 5F-AMB,	PM: severe (non-cardiogenic) pulmonary edema, hypoxic	Maeda et al. (2017)

(continued)

Table 2 (continued)

Substance	CoD	N	C blood (ng/mL)	Antemortem and postmortem findings	References
			FUB-PB-22, and AB-FUBINACA	encephalopathy, and systemic hypoxia; seizures suggested by the authors	
	Sudden cardiac death by dilated cardiomyopathy as a result of synthetic cannabinoid intoxication	1	AB-CHMINACA: 8.2	Dilated cardiomyopathy, cardiomegaly, bilateral pulmonary edema, bilateral pleural effusion, and ascites; cardiomyocyte hypertrophy, contraction band necrosis, pulmonary edema, and pulmonary vascular congestion	Paul et al. (2017)
	Not stated	6	AB-CHMINACA confirmed		Trecki et al. (2015)
AB-FUBINACA	Not stated	1	AB-FUBINACA confirmed		Trecki et al. (2015)
ADB-FUBINACA	Coronary arterial thrombosis in combination w/ADB-FUBINACA use	1	ADB-FUBINACA: 7.3	AM: violent, aggressive behavior after smoking, unresponsive shortly after; PM: pulmonary edema, vascular congestion and thrombotic occlusion of left coronary artery, and ischemia of left myocardium	Shanks et al. (2016)
	Not stated	1	ADB-FUBINACA confirmed		Trecki et al. (2015)
CUMYL-4CN-BINACA	Intoxication	6	CUMYL-4CN-BINACA: 0.4–11.9		Yeter (2017)

Polydrug intoxications involving synthetic cannabinoid receptor agonists					
JWH-122, AM-2201, MAM-2201, UR-144, and amphetamine	Intoxication w/ several SCRAs and amphetamine as (at least) contributory CoD	1	JWH-122: 0.39, MAM-2201: 1.5, AM-2201: 1.4, UR-144: 6.0, and JWH-018: 0.1 (all femoral)	PM: stenosing coronary sclerosis, pulmonary and cerebral edema, and congestion of inner organs	Schaefer et al. (2013)
AB-CHMINACA, AB-FUBINACA, 5F-AMB, 5F-AKB-48, STS-135, THJ-2201	Diabetic ketoacidosis – unclear whether the consumed SC produced hyperglycemia or whether victim forgot insulin administration	1	AB-CHMINACA: 2.8, AB-FUBINACA: 0.97, 5F-AMB: 0.19, 5F-AKB-48: 0.51, STS-135: 0.16, and THJ-2201: 0.16 (all femoral)	Additional PM findings: acetone 163 mg/L and 336 mg/L (femoral/heart blood); HbA1c 98 mmol/mL (femoral); glucose 299 mg/dL, and lactate 164 mg/dL (vitreous humor)	Hess et al. (2015)
AB-CHMINACA, 5F-AMB, and diphenidine	Intoxication mostly by diphenidine, and contribution of AB-CHMINACA and 5F-AMB	1	AB-CHMINACA: ND in blood; brain: 15.6 ng/g, heart muscle: 20 ng/g, and adipose tissue: 24.8 ng/g 5F-AMB: 18.7 ng/g (adipose tissue) Diphenidine: 715 ng/g (femoral) and 707 ng/g (heart blood)		Hasegawa et al. (2015); Wurita et al. (2016)
5F-ADB, ADB-CHMINACA, and 5F-ADB-PINACA	Asphyxia, SCRA poisoning as indirect CoD	1	5F-ADB: stomach 3.18 ng/g; brain: 1.9 ng/g; heart muscle: 1.82 ng/g; spleen: 1.17 ng/g; pancreas: 1.61 ng/g; adipose tissue: 7.95 ng/g; and ADB-CHMINACA: 6.05 (femoral), 10.6 (cardiac) 5F-ADB-PINACA identified in herbal blends	PM: trachea filled with stomach contents indicating lowered consciousness and vomiting before death	Hasegawa et al. (2015)

(continued)

Table 2 (continued)

Substance	CoD	N	C blood (ng/mL)	Antemortem and postmortem findings	References
MAM-2201, AM-1220, and AM-2232	"Misuse of these synthetic cannabinoids more or less contributed to the victim's death"	1	MAM-2201: 16.3 AM-1220: 140 AM-2232: 0.86 (all femoral) Concentrations 1.5–5.5 x higher in heart blood suggesting PM redistribution		Zaitsu et al. (2015)
AM-2201, JWH-122, and JWH-210		1	SCRAs confirmed		Trecki et al. (2015)
UR-144/XLR-11		1	SCRAs confirmed		Trecki et al. (2015)
THJ-2201/AB-PINACA		1	SCRAs confirmed		Trecki et al. (2015)
AB-CHMINACA, AB-PINACA, ADB-PINACA, and ADB-CHMINACA		1	SCRAs confirmed		Trecki et al. (2015)
AB-CHMINACA, AKB-48, and XLR-11		1	SCRAs confirmed		Trecki et al. (2015)
AM-694, AM2201, and JWH-018 w/ methoxetamine	Intoxication w/ methoxetamine, contribution of three SCRAs cannot be ruled out	1	Methoxetamine: 8,600 ng/g (femoral) AM-694: 0.09 ng/g and AM-2201 0.3 ng/g JWH-018 0.05 ng/g	Pulmonary edema	Wikstrom et al. (2013)
UR-144, XLR-11, and JWH-022	Sudden death associated with synthetic cannabinoid intoxication	1	UR-144: 12.3, XLR-11: 1.3, and JWH-022: 3.0	PM: no remarkable findings	Paul et al. (2017)

AM antemortem, *PM* postmortem, *M* male, *F* female, *ND* not detected, *SCRA* synthetic cannabinoid(s), *CoD* cause of death

[a]Eighteen cases in total listed in the paper. JWH-018 concentrations for the remaining 15 ranged between 0.1 and 5.5 μg/L. The authors suggested postmortem redistribution for the cases with high concentrations

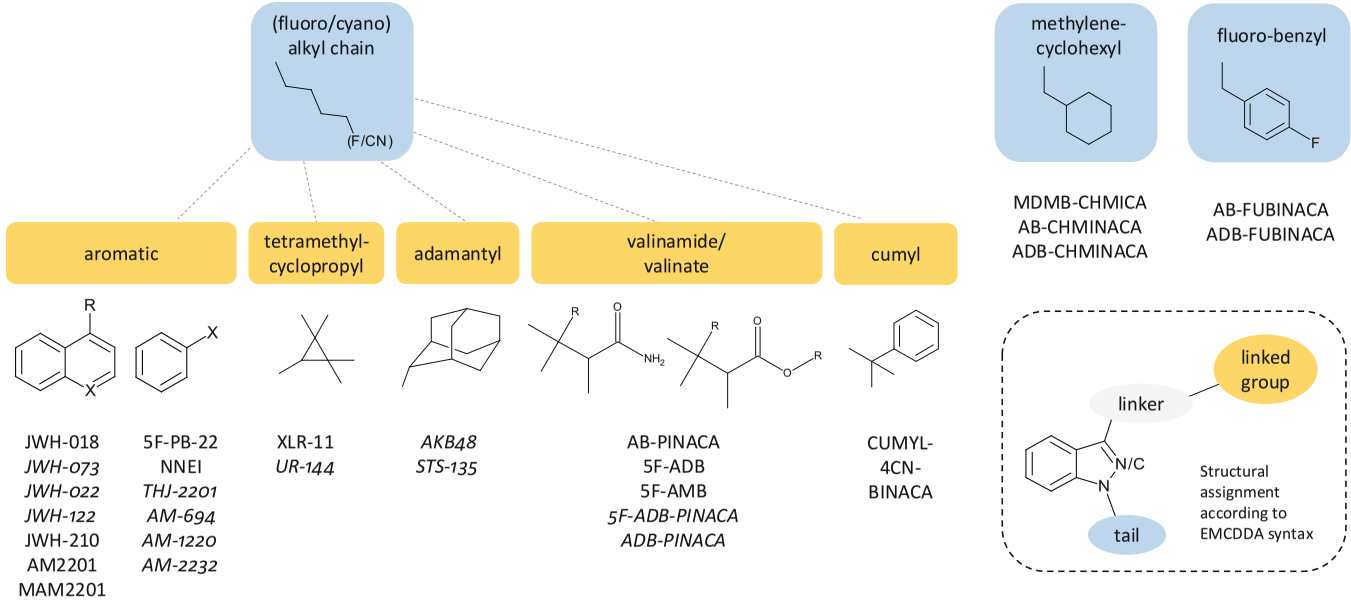

Fig. 1 Synthetic cannabinoid receptor agonists (SCRAs) involved in fatal intoxication cases. Chemical structures are organized by their respective structural elements: first by tail (blue), then by the linked group (yellow). Names are written in italics in cases where the SCRA was only found in a mixed intoxication case

all. In case of the latter, the presence of an SCRA in body tissues or blood can be the only remaining explanation for the death in the light of the circumstances. Despite all ambiguities related to the presented cases, there is one striking observation: many antemortem adverse effects as well as postmortem findings can be related to the heart. Several intoxicated patients experienced chest pain, trouble breathing, low pulse, cardiac arrest, and asystole; postmortem findings either indicated a possible preexisting heart disease or possible cardiotoxicity (enlarged heart, arteriolar wall hypertrophy, ischemic cardiac disease, heart fibrosis, myocardial cell death, heart failure, etc.). The underlying mechanisms that lead to this severe toxicity or contributing factors that might worsen their effects essentially remain unclear at the moment. Sudden onset cardiac dysrhythmias, severe seizures, or liver toxicity (possibly including a role via metabolites) have been suggested as potential mechanisms of action (Behonick et al. 2014; Maeda et al. 2017).

Table 2 lists the concentrations of the respective SCRA found in the blood of the decedents. However, at this point, no lethal concentrations can be defined due to the following two reasons: (1) development of individual tolerance: as has been reported by several groups, antemortem and postmortem concentrations of SCRAs can overlap significantly. In other words, a concentration found to be lethal for one person might not result in harms in another case (Angerer et al. 2016); (2) interpretation issues inherent to postmortem blood concentrations: it has been shown that due to their lipophilic nature SCRAs are redistributed postmortem (Zaitsu et al. 2015), which creates major difficulties in assessing blood concentrations at the time of death and in establishing any reference values.

A few compounds deserve a closer look as they were involved in a series of fatalities and/or were identified by several laboratories. Furthermore, a number of cases are worth highlighting, either because their circumstances were somewhat peculiar or because the level of detail describing case history and ante- and postmortem findings stand out.

JWH-018 is the prototypical pentylindole SCRA and was among the first popular compounds on the market. JWH-073 is the butyl chain analog of JWH-018. Shanks et al. (2012) published a case series mentioning a total of 18 fatalities with JWH-018 and JWH-073 concentrations between 0.1–199 ng/mL and 0.1–68.3 ng/mL, respectively. The authors described three cases with extremely high JWH-018 concentrations (19.6, 83.3, and 199 ng/mL in cardiac blood) in more detail and proposed that postmortem redistribution had occurred.

Behonick et al. (2014) reported on four fatalities involving 5F-PB-22, a quinolinyl SCRA with an ester linker. All victims were young (17–27 years) and in all cases, the circumstances strongly suggested that 5F-PB-22 consumption actively contributed to death. Three of the victims died suddenly after partying and smoking 5F-PB-22, the fourth one expired within 2 days after a rapidly deteriorating hospital course. 5F-PB-22 blood concentrations were in a similar low range (1.1–1.5 ng/mL) detected in the three postmortem and one antemortem blood samples. Angerer et al. (2017) published a case with 0.37 ng/mL 5F-PB-22 and 2.6 g/kg ethanol in femoral blood, where the cause of death was ruled due to suffocation in the presence of 5F-PB-22 and ethanol; both may have contributed to the fatal outcome.

Several case reports (Angerer et al. 2017; Kusano et al. 2017; Usui et al. 2017) documented fatalities involving 5F-ADB, also known as 5F-MDMB-PINACA, an indazole-based SCRA with a methylvalinate moiety as the linked group. In all cases, blood concentrations for 5F-ADB were low (<2 ng/mL) and the circumstances, such as the positions of the victims ("found holding a cigarette lighter" and "found in his car lying in the front passenger seat and holding a plastic pipe") and the drug paraphernalia on site ("opened sachet on site," "opened sachet found," and "unsealed package") suggested that death had occurred abruptly and shortly after consumption. In the majority of cases, there were no remarkable findings at autopsies leaving 5F-ADB the most likely cause of death. If any, 5F-ADB would fulfill the criteria to be considered the most toxic candidate among the SCRAs found in this data compilation.

AB-CHMINACA, an indazole synthetic cannabinoid carrying a methylenecyclohexyl tail and valinamide as linked group, was detected in fatal cases by at least five laboratories (Angerer et al. 2017; Gieron and Adamowicz 2016; Maeda et al. 2017; Paul et al. 2017; Trecki et al. 2015). Similar to scenarios observed in other cases, the victims were either found in life-threatening conditions (cardiopulmonary arrest; collapse, unconsciousness, and trouble breathing) after consumption of AB-CHMINACA or dead in a position suggesting sudden death during smoking ("found kneeling in front of his sofa holding a bong and a lighter in his hands"). AB-CHMINACA concentrations were low (1.5–8.2 ng/mL); in one case, trace amounts of 5F-AMB, FUB-PB-22, and AB-FUBINACA were also observed and in two cases significant but nonlethal ethanol levels were detected.

Two cases involved a longer hospital stay demonstrating that even immediate treatment cannot always guarantee a favorable outcome. In these settings, antemortem samples were available for analysis providing information about the blood concentration of the synthetic cannabinoids before death. In the first case, already mentioned above, a 27-year-old male consumed 5F-PB-22 and was treated for 2 days after being discovered "quite ill and diaphoretic" and diagnosed with severe liver injury, severe coagulopathy, acute kidney injury, acute respiratory failure, hypoxemia, severe anion gap metabolic, and lactic acidosis (Shanks et al. 2012). He experienced a brief episode of cardiac arrest and his clinical condition deteriorated due to circulatory failure, respiratory failure, CNS failure, renal failure, and severe metabolic derangement. At autopsy, fulminant liver failure was ruled cause of death. In the second case, the decedent consumed MDMB-CHMICA (Adamowicz 2016), an SCRA with a methylenecyclohexyl tail and valinate as the linked group. Moments after smoking, the 25-year-old man fell to the floor, vomited, and lost consciousness; 30 min later at the emergency department, he had no pulse and scored 3 on the Glasgow Coma Scale. He died 4 days later after two cardiac arrests. Multiple organ failure (respiratory, circulatory, heart, kidney, and liver) and hypoxic-ischemic damage of the CNS were determined as cause of death. The MDMB-CHMICA blood concentration antemortem was 5.6 ng/mL.

In two cases, the victim was assumed to have experienced a psychiatric episode after SCRA consumption, which resulted in suicide (Shanks et al. 2012) or self-inflicted stabbing (Patton et al. 2013). JWH-018 at a concentration of 83.3 ng/mL

and AM2201 at a concentration of 12 ng/mL were found in postmortem blood, respectively. In a third case, in which a synthetic cannabinoid was considered indirectly responsible for the death, the deceased had smoked JWH-210 with a friend and was later found dead outdoors having died from hypothermia (Kronstrand et al. 2013a).

Finally, one fatality stands out where diabetic ketoacidosis was ruled cause of death but the contribution of SCRAs was suggested (Hess et al. 2015). The decedent was found dead at home and was known to be a drug consumer and suffering from insulin-dependent diabetes. At autopsy, not only six synthetic cannabinoids were identified (AB-CHMINACA 2.8 ng/mL, AB-FUBINACA 0.97 ng/mL, 5F-AMB 0.19 ng/mL, 5F-AKB-48 0.51 ng/mL, STS-135 0.16 ng/mL, and THJ-2201 0.16 ng/mL in femoral blood), but also acetone (163 mg/L) and elevated HbA1c (98 mmol/mL) were observed. High glucose (299 mg/dL) and lactate (164 mg/dL) concentrations in vitreous humor indicated high glucose levels antemortem. Since SCRAs have been shown to induce hyperglycemia, the question remained whether the decedent had skipped insulin injections or whether the SCRAs led to a hyperglycemic state.

5 Synthetic Cathinones and Phenethylamines

Ring-substituted synthetic cathinones and a range of ring-substituted phenethylamines typically produce sympathomimetic and hallucinogenic toxidromes. A multitude of complications related to the cardiovascular, neurological, respiratory, and gastrointestinal systems have also been reported (Adebamiro and Perazella 2012; Borek and Holstege 2012; Boulanger-Gobeil et al. 2012; Derungs et al. 2011; Garrett and Sweeney 2010; Luciano and Perazella 2014; Penders 2013; Penders and Gestring 2011; Penders et al. 2012a, b; Thornton et al. 2012; Warrick et al. 2012; Wood et al. 2010, 2011, 2013). The hallucinogenic toxidrome includes disorientation, hallucinations, psychotic episodes, paranoia, anxiety, and memory disruption in combination with cardiovascular complications such as hypertension, tachycardia, and tachypnea. The sympathomimetic toxidrome includes anxiety, paranoia, delusions, and hyperactivity together with hyperpyrexia, diaphoresis, mydriasis, and seizures as well as hypertension and tachycardia. In addition, serotonin syndrome and excited delirium have been reported (Andreasen et al. 2015; Bersani et al. 2014; Murray et al. 2012; Penders 2013; Penders and Gestring 2014; Penders et al. 2012a; Vevelstad et al. 2012). In contrast to the model substances amphetamine and cathinone, there are numerous reports of fatal intoxications related to synthetic cathinones and phenethylamines. In the following paragraphs and in Table 3, information from deaths attributed to these compounds is summarized.

Table 3 Summary of the case report data of cathinone or phenethylamine poisonings

Substance	CoD	N	Administration	C blood (ng/mL)	Antemortem and postmortem findings	References
3,4-DMMC	Intoxication	1	Injection	27,000	Needle marks, congestion of abdominal organs, and pulmonary edema	Usui et al. (2014)
MDPV	Intoxication	1	Oral	1,000	AM: agitation, tachycardia, and hyperthermia	Kesha et al. (2013)
					PM: unremarkable	
MDPV	Intoxication	1	Injection/ snorting	82	Agitation, delusional, violent, dilated pupils, tachycardia, bradycardia, cardiac arrest, hyperthermia, rhabdomyolysis, hyperkalemia, metabolic acidosis, anoxic brain injury, and brain death (support withdrawn)	Murray et al. (2012)
MDPV	Intoxication	1	Snorting	440	Pulmonary edema and mildly enlarged heart	Wyman et al. (2013)
MDPV	Natural causes and drug abuse	2		39 and 130	Diabetic ketoacidosis and pneumonia	Wright et al. (2013)
Mephedrone	Intoxication	1		5,500	Brain stem failure and lung injury	Adamowicz et al. (2013)
Mephedrone	Mixed intoxication	1	Oral	500	AM: unconsciousness	Aromatario et al. (2012)
					PM: bilateral myocardial hypertrophy, pulmonary edema, and hepatomegaly	
Mephedrone	Intoxication	2		1940 (am) and 2,100	–	Cosbey et al. (2013)
Mephedrone	Mixed intoxication	2		40 and 220	Pulmonary edema	Cosbey et al. (2013)
Mephedrone	Mixed intoxication	1		1,330	Pulmonary edema and multi-visceral congestion	Gerace et al. (2014)
Mephedrone	Intoxication	1	Oral	5,100	AM: agitation	Lusthof et al. (2011)
					PM: minor brain swelling and pulmonary edema	

(continued)

Table 3 (continued)

Substance	CoD	N	Administration	C blood (ng/mL)	Antemortem and postmortem findings	References
Mephedrone	–	4		1,200–22,000	–	Torrance and Cooper (2010)
Mephedrone	Intoxication	1	Insufflation	980	AM: vomiting	Maskell et al. (2011)
					PM: coronary atherosclerosis and myocardial fibrosis	
Mephedrone	Intoxication	1	Oral	2,240	AM: shaking, twitching, sweating, and cardiopulmonary arrest	Maskell et al. (2011)
					PM: –	
Mephedrone	Mixed intoxication	1	Oral	130	AM: slow and heavy breathing	Maskell et al. (2011)
					PM: coronary atherosclerosis	
Mephedrone	Trauma	1		230	Blunt force injuries	Maskell et al. (2011)
Methedrone	Intoxication	1		9,600	AM: unconsciousness, seizures, and asystole	Wikstrom et al. (2010)
					PM: pulmonary edema and congestion	
Methedrone	Intoxication	1	Oral	8,400	Unconsciousness, hyperthermia, and complete organ failure	Wikstrom et al. (2010)
Methylone	Intoxication	1		670	–	Cawrse et al. (2012)
Methylone	Intoxication	1		840	Hyperthermia, rhabdomyolysis, acute renal failure, seizures, metabolic acidosis, and cardiac arrest	Pearson et al. (2012)
Methylone	Intoxication	1	Oral	3,300	Seizures and asystole	Pearson et al. (2012)
Methylone	Intoxication	1		0560	Irrational behavior, sweating, hyperthermia, and metabolic acidosis	Pearson et al. (2012)
Methylone	Drowning	1		3,400	Found floating face down in water	McIntyre et al. (2013)

Methylone	Intoxication	1		0700	AM: sudden collapse and cardiopulmonary arrest	Carbone et al. (2013)
					PM: pulmonary congestion and edema	
Methylone	Intoxication	1	Oral	3,130	AM: breathing difficulties, polypnea, and cardiopulmonary arrest	Barrios et al. (2016)
					PM: anoxia, pulmonary congestion	
Methylone and butylone	Mixed intoxication	1	Oral	Present in urine	AM: seizures, hyperthermia, tachycardia, tachypnea, disseminated intravascular coagulation, and coma	Warrick et al. (2012)
					PM: generalized coagulopathy, fatty liver, and anoxic encephalopathy	
N-Ethylnorpentylone	Intoxication	1		Present	AM: agitation, disorientation, hypotension, tachycardia, hyperthermia, cardiac arrest, extreme acidosis, disseminated intravascular coagulation, rhabdomyolysis, and acute kidney injury	Thirakul et al. (2017)
					PM: cerebral edema, pulmonary edema, mildly enlarged heart, and left ventricular hypertrophy	
Pentedrone and alpha-PVP	Mixed intoxication	1	Oral	8,790 and 900	AM: cardiac arrest	Sykutera et al. (2015)
					PM: pulmonary edema, atherosclerosis, and chronic changes in the heart	
Alpha-PVP	Intoxication	1		410	AM: agitation and collapse	Nagai et al. (2014)
					PM: severe organ congestion and severe myocardial contraction bands	
Alpha-PVP	Intoxication	1		174	AM: cardiac arrest, circulatory insufficiency, and metabolic acidosis	Potocka-Banas et al. (2017)
					PM: cerebral and pulmonary edema, and hypertrophy and scarring of the heart	
5-IT	Intoxication	5		1,000–4,200	PM: unremarkable	Kronstrand et al. (2013b)
5-IT	Mixed intoxication	6		700–18,600	PM: unremarkable	Kronstrand et al. (2013b)

(continued)

Table 3 (continued)

Substance	CoD	N	Administration	C blood (ng/mL)	Antemortem and postmortem findings	References
5-IT	Intoxication	1		1,200	Hyperthermia, shallow breathing, fixed and dilated pupils, and extensive confluent hemorrhage of right lung	Seetohul and Pounder (2013)
5-IT	Mixed intoxication	1		1,000	PM: unremarkable	Seetohul and Pounder (2013)
5-IT	Mixed intoxication	1		500	AM: sweating, slurred speech, and cardiac arrest PM: unremarkable	Seetohul and Pounder (2013)
5-IT	Mixed intoxication	1		400	AM: sweating and agitation PM: lung edema and congestion, and white foam in trachea and bronchi	Seetohul and Pounder (2013)
PMA	Intoxication	1	Oral	2,347	PM: damage to important centers of the brain stem	Gil et al. (2013)
PMMA	Intoxication	3		1,580–3,300	PM including: lung edema and glomerulosclerosis	Vevelstad et al. (2012)
PMMA	Mixed intoxication (stimulants)	6		1,520–3,230	PM including: respiratory distress, hyperthermia, pulmonary congestion, multi-organ failure, hyperkalemia, intravascular coagulation, hypoglycemia, lung edema, petechial bleeding in gastric mucosa, and cardiac hypertrophy	Vevelstad et al. (2012)
PMMA	Mixed intoxication (CNS depressants)	3		170–1,240	PM including: pulmonary congestion and sparse bleeding in subcutaneous tissues	Vevelstad et al. (2012)
PMMA	Intoxication	7		1,010–5,100	–	Lurie et al. (2012)
PMMA	Mixed intoxication	14		80–6,020	–	Lurie et al. (2012)

25I-NBOMe		1	Oral	Present	AM: hallucinations, flailing, agitation, and unresponsiveness	Walterscheid et al. (2014)
					PM: subcutaneous hemorrhages, scattered petechiae on epicardial surface, aspiration of gastric contents, and moderate lung edema and congestion	
25I-NBOMe		1	Oral	Present	AM: unwellness, flailing, and unresponsiveness	Walterscheid et al. (2014)
					PM: white foam in trachea and bronchi, and moderate lung congestion	
25I-NBOMe	Trauma	1		0.4	AM: paranoia and bizarre behavior	Poklis et al. (2014)
					PM: multiple impact injuries (fall)	
25I-NBOMe	Intoxication	1	Oral	0.76 (AM)	AM: vomiting, convulsions, acute renal failure, liver failure, and intravascular coagulation	Lowe et al. (2015)
					PM: unremarkable	
25I-NBOMe	Intoxication	1	Insufflation	28	AM: agitation, seizures, and vomiting	Kueppers and Cooke (2015)
					PM: pulmonary congestion	
25C-NBOMe	Mixed intoxication	1	Insufflation	Present	Generalized seizures, unconsciousness, acute renal failure, and metabolic acidosis	Tarpgaard et al. (2015)
25C-NBOMe	Mixed intoxication	1	Insufflation	0.6	AM: agitation, hallucinations, convulsions, hyperthermia, respiratory and metabolic acidosis, rhabdomyolysis, and multi-organ failure	Andreasen et al. (2015)
					PM: organ congestion, diffuse mucosal hemorrhage, and liver degeneration	
25B-NBOMe	Intoxication	1	Oral/buccal	1.6	AM: destructive behavior and collapse	Shanks et al. (2015a)
					PM: blunt force trauma, lacerations, contusions, abrasions, severe pulmonary edema, and aspiration	
25I-NBOMe	Intoxication	1		Present urine	PM: multiple contusions and abrasions, severe pulmonary edema, and mild cerebral edema	Shanks et al. (2015a)
Methylone and butylone	Mixed intoxication	1	Oral	Present urine	AM: seizures, hyperthermia, tachycardia, tachypnea, disseminated intravascular coagulation, and coma	Warrick et al. (2012)

(continued)

Table 3 (continued)

Substance	CoD	N	Administration	C blood (ng/mL)	Antemortem and postmortem findings	References
					PM: generalized coagulopathy, fatty liver, and anoxic encephalopathy	
N-Ethylnorpentylone	Intoxication	1		Present	AM: agitation, disorientation, hypotension, tachycardia, hyperthermia, cardiac arrest, extreme acidosis, disseminated intravascular coagulation, rhabdomyolysis, and acute kidney injury	Thirakul et al. (2017)
					PM: cerebral edema, pulmonary edema, and mildly enlarged heart and left ventricular hypertrophy	
Pentedrone and Alpha-PVP	Mixed intoxication	1	Oral	8,790 and 900	AM: cardiac arrest	Sykutera et al. (2015)
					PM: pulmonary edema, atherosclerosis, and chronic changes in the heart	
Alpha-PVP	Intoxication	1		410	AM: agitation and collapse	Nagai et al. (2014)
					PM: severe organ congestion and severe myocardial contraction bands	
Alpha-PVP	Intoxication	1		174	AM: cardiac arrest, circulatory insufficiency, and metabolic acidosis	Potocka-Banas et al. (2017)
					PM: cerebral and pulmonary edema, and hypertrophy and scarring of the heart	
5-APB	Intoxication	1		2,500	AM: breathing difficulties, and cardiac and pulmonary arrest	McIntyre et al. (2015a)
					PM: foam in trachea, and pulmonary congestion and edema	
5-APB and 3-MMC	Mixed intoxication	1	Oral	5,600 and 1,600	AM: agitation, seizures, hyperthermia, tachycardia, tachypnea, increased blood pressure, and cardiac arrest	Adamowicz et al. (2014)
					PM: no information available?	

AM antemortem, *PM* postmortem, *CoD* cause of death

5.1 Mephedrone

According to the EMCDDA, mephedrone made its first appearance in the European Union (EU) during late 2007 when Finnish customs seized a white powder (EMCDDA-Europol 2010). In Sweden, mephedrone was encountered during 2008 and 2009, but gradually disappeared after extensive media coverage related to some acute overdoses and scheduling as a narcotic (Wikstrom et al. 2010). Most of the European countries have reported its use between 2008 and 2010. In a 2009 survey, Winstock et al. (2011) reported on the use of mephedrone in the UK documenting undesired effects that included sweating, heart race, restlessness, overheating, tremor, panic, agitation, headache, shortness of breath, and blurred vision, which were reported by more than 30% of the survey participants. Case reports from fatal intoxications with mephedrone are commonly accompanied by unremarkable findings from autopsies and with little information about the antemortem symptoms (Adamowicz et al. 2013; Aromatario et al. 2012; Cosbey et al. 2013; Gerace et al. 2014; Lusthof et al. 2011; Maskell et al. 2011; Torrance and Cooper 2010). Cosbey et al. reported quite different concentrations of mephedrone in cases involving monointoxications (1.94 and 2.10 μg/mL) and in mixed intoxications (0.04 and 0.22 μg/mL) and some cases with other causes of death (0.004–0.65 μg/mL) (Cosbey et al. 2013). This is in accordance with a paper published by Loi et al. (2015), who concluded that most cases were multiple intoxications of mephedrone with other drugs present (87%) or contributing (60%) to the fatal outcome and that the combination with other stimulant drugs with mephedrone can, in a synergistic manner, increase the dopaminergic and serotonergic stimulation causing more severe adverse effects.

5.2 Methylone

Methylone appeared around 2004 and has been reported as the cause of death in several cases from 2012 and onwards (Barrios et al. 2016; Carbone et al. 2013; Cawrse et al. 2012; McIntyre et al. 2013; Pearson et al. 2012). In their report documenting three fatal intoxications, Pearson et al. (2012) reported all patients to present with seizure-like activity and hyperthermia prior to death. Two cases exhibited metabolic acidosis and one hospitalized patient also developed rhabdomyolysis, acute renal failure, and acute respiratory failure before going into cardiac arrest. The authors suggested that at blood concentrations above 0.5 μg/mL, methylone could be lethal. In a recent case reported by Barrios et al. (2016), a patient showed a postmortem peripheral blood concentration of 2.13 μg methylone/mL blood. The autopsy revealed anoxia and pulmonary congestion suggesting adverse effects related to the pulmonary system and the cause of death was considered respiratory distress.

5.3 MDPV

MDPV has been present on the European drug market since at least 2007 when its first identification in products was reported by Westphal et al. (2009). The first report to the EMCDDA, though, was not until late 2008 reported by Finland (EMCDDA–Europol 2009). The prevalence in the USA increased during 2010 as reported by Spiller et al. (2011). However, there are few analytically confirmed reports of fatal toxicity in the scientific literature published after 2010 (Kesha et al. 2013; Murray et al. 2012; Wright et al. 2013; Wyman et al. 2013). Borek and Holstege described that despite intensive care MDPV intake may cause multiorgan failure from direct cellular toxicity or from the severe hyperthermia (Borek and Holstege 2012). In addition, excited delirium has been mentioned in reports of fatal MDPV intoxication (Kesha et al. 2013; Murray et al. 2012). Excited delirium is a serious medical condition associated with acute onset of agitated violent behavior that often culminates in a sudden unexplained death with physical signs of hyperthermia, tachypnea, and tachycardia (Mash et al. 2009).

In their case report from 2012, Murray et al. (2012) describe a patient who, after injecting and snorting MDPV, became aggressive, delusional, removed his clothes, and ran outside. When apprehended by the police, he showed aggression and considerable strength and violent behavior, which persisted as he was taken to the hospital by ambulance. Upon arrival, he immediately developed bradycardia and subsequent cardiac arrest but was resuscitated and put on life support and medical treatments. Over the next hours, he developed hyperthermia, hyperkalemia, metabolic acidosis, oliguric renal failure, and rhabdomyolysis. In addition, an "edema and early anoxic brain injury" was suspected. His neurologic values worsened and 42 h after admission the patient was declared brain dead.

5.4 Alpha-PVP

Seizure data reported to EMCDDA (EMCDDA-Europol 2012) suggest that alpha-PVP has been present on the EU drug market since at least 2011. Alpha-PVP has been analytically confirmed in 116 deaths according to the EMCDDA risk assessment report (EMCDDA 2015). In 23 cases, alpha-PVP was considered the cause of death or contributing to death. Apart from that report, there are few reports on acute fatal intoxications involving alpha-PVP (Hasegawa et al. 2014a; Nagai et al. 2014; Potocka-Banas et al. 2017; Sykutera et al. 2015). Two reports (Nagai et al. 2014; Sykutera et al. 2015) describe postmortem findings of hemosiderin-laden pulmonary macrophages compatible with heart failure. Histology revealed changes in the heart tissue such as severe myocardial contraction bands. Autopsy results also included pulmonary edema. The blood concentrations of alpha-PVP were 901 and 411 ng/g. In a third case reported by Potocka-Banas et al., a male patient was admitted to intensive care with cardiac arrest (Potocka-Banas et al. 2017). The concentration of alpha-PVP in blood was 174 ng/mL and no other drugs were found. The patient was acidotic and his status deteriorated dramatically with circulatory insufficiency and

arrhythmias, which resulted in death after 5 h. Significant autopsy findings included cerebral and pulmonary edema and generalized congestion of internal organs. Hypertrophy and scarred heart tissue were observed and, based on these and the toxicological findings, death was attributed to intoxication with alpha-PVP with subsequent cardiac arrest.

5.5 5-IT

5-(2-Aminopropyl)indole (5-IT) has been available as a recreational drug since at least 2011 and its detection was first reported to the EMCDDA in 2012 (EMCDDA-Europol 2013). In their risk assessment report (EMCDDA 2013a), the EMCDDA counted 24 deaths associated with 5-IT up to 2012. 5-IT is a positional isomer of alpha-methyltryptamine (AMT) but considered an amphetamine derivative due to presence of the phenylisopropylamine side chain at the 5-position of the indole ring. The acute toxicity of 5-IT includes symptoms consistent with monoaminergic toxicity. In addition, there is a possibility of interactions with other substances, including medicinal products and stimulants, that act on the monoaminergic system increasing the risk of serotonin syndrome (EMCDDA 2013a). In a series of analytically confirmed intoxications reported by Bäckberg et al., clinical symptoms of sympathomimetic toxicity were the most common findings (Bäckberg et al. 2014). These included tachypnea, tachycardia, dilated pupils, hypertension, agitation, and increased perspiration. In addition, rhabdomyolysis and hyperkalemia were seen in five and three out of the 14 patients. Serum samples were analyzed for 5-IT with concentrations ranging from 0.01 μg/mL to 0.59 μg/mL ($N = 8$) with a median of 0.22 μg/mL. In comparison to reported deaths attributed to 5-IT, these concentrations seemed to be rather low. However, it is not known whether the patients would have survived without hospital care. In a series of deaths reported by Kronstrand et al., all but one of the 15 victims were found dead at home (Kronstrand et al. 2013b). Similar scenarios were described by Seetohul et al. (2013) with four fatalities occurring outside the hospital. The concentrations of 5-IT ranged between 0.7 and 18.6 μg/g femoral blood (Kronstrand et al. 2013b) and between 0.4 and 1.2 μg/mL (Seetohul and Pounder 2013). Seetohul suggested that the principal mechanism of death in their four fatalities was acute cardiac failure.

5.6 PMMA and PMA

PMMA and PMA are the *para*-methoxylated analogs of methamphetamine and amphetamine. Common symptoms of PMMA and PMA intoxication include hyperactivity, hallucinations, muscle spasms, convulsions, hyperthermia, rhabdomyolysis, bradycardia, tachycardia, cardiac arrest, and multiorgan failure (Johansen et al. 2003; Lin et al. 2007; Martin 2001; Vevelstad et al. 2012). Since the early 2000s, there have been outbreaks of PMMA and PMA use leading to deaths as shown by papers from Denmark, Canada, and Taiwan (Johansen et al. 2003; Lin

et al. 2007; Martin 2001). Common autopsy findings included pulmonary edema, multiple organ congestion, pulmonary hemorrhage, brain edema as well as subarachnoid and subdural hemorrhage. Also, renal tubular and hepatic necrosis and rhabdomyolysis have been reported. In a recent report from Norway, Vevelstad and co-workers (2012) gave a thorough insight into both fatal and nonfatal intoxications from PMMA intake. They showed low or moderate PMMA concentrations in whole blood from the nonfatal intoxications but much higher concentrations in the fatal cases, especially those when no other drugs were found together with PMMA/PMA. The symptoms and the cause of death from PMMA and PMA intake appeared to be consistent with the serotonin syndrome. In addition to increasing the levels of serotonin by blocking the 5HT-transporter, PMA and PMMA are potent MAO-A inhibitors (Callaghan et al. 2005; Simmler et al. 2014). This increases the risk of precipitating the serotonin syndrome and may lead to cardiovascular toxicity (Vevelstad et al. 2012).

5.7 NBOMes

The first reports about the appearance of *N*-(2-methoxybenzyl)phenethylamine derivatives (NBOMe compounds) date back to 2010 (Zuba et al. 2013). Typically, these drugs are *N*-(2-methoxybenzyl) derivatives of 2,4,5-ring-substituted phenethylamines. This particular modification dramatically increases the affinity and functional activity at the $5HT_{2A}$ receptor, which is thought to be a key mediator in hallucinogenic effects whereas agonist action at alpha-adrenergic receptors can mediate stimulant effects (Rickli et al. 2015). The stimulant effects may be responsible for the cardiovascular toxicity but animal studies have also shown that blocking of the 5HT receptors rather than the adrenergic receptors reduced the cardiovascular effects of the hallucinogen DOI (Alper 1990). There are few data about the toxicity of NBOMes in the scientific literature but case reports of fatal intoxication are available (Andreasen et al. 2015; Kueppers and Cooke 2015; Lowe et al. 2015; Poklis et al. 2014; Shanks et al. 2015a). Typical symptoms of NBOMe intoxication were related to serotonin and sympathomimetic toxidromes (Bersani et al. 2014; Halberstadt 2017). Commonly, patients present with confusion, agitation, convulsions, and seizures together with hypertension, tachycardia, hyperthermia, rhabdomyolysis, acidosis, and multiple organ failures, which might lead to death. Kueppers and Cooke described a case, which involved several NBOMes where 25I-NBOMe was found at a concentration of 28 ng/mL blood and the analogs 25H-NBOMe and 25C-NBOMe at significantly lower concentrations of 1 ng/mL and 0.7 ng/mL, respectively (Kueppers and Cooke 2015). Shortly after administration by nasal insufflation, the patient became agitated, experienced seizures, and vomited before collapsing. CPR was unsuccessful. Autopsy revealed pulmonary congestion and microscopic evaluation confirmed edema and features in agreement with agonal aspiration. Similar presentations with agitation, vomiting, and sudden collapse followed by death were seen in an intoxication with 25B-NBOMe (Shanks et al. 2015a). Andreasen et al. reported a fatal intoxication with 25C-NBOMe and

amphetamine with typical signs of serotonin toxicity prior to death (Andreasen et al. 2015). Despite intensive care, the patient continued to have hyperkalemia, low blood pressure, and increasing coronary markers and his condition deteriorated with disseminated intravascular coagulation. The antemortem and postmortem concentrations of 25C-NBOMe were 0.81 ng/g and 0.60 ng/g blood, respectively. The postmortem amphetamine concentration was 470 ng/g and the cause of death was attributed to the untoward effects of 25C-NBOMe and amphetamine.

6 Miscellaneous Drugs

NPS are divided into groups based either on their chemical structure (cathinones and phenethylamines), or grouped based on their effects (SCRAs and MOR agonists). However, there are a number of substances that make classifications more difficult. Nevertheless, they have proven to be the cause of fatalities in the 2010s.

6.1 BZP and TFMPP

1-Benzylpiperazine (BZP), also known as "A2," and 1-(3-trifluoromethylphenyl) piperazine (TFMPP), also known as "Molly" at that time, were two piperazine derivatives often found together, presumably reflecting marketing efforts but also pharmacological similarities. Baumann et al. (2005) studied the release of radiolabeled substrates from synaptosomes and showed that BZP increased the release mediated by the dopamine transporter in a manner similar to MDMA whereas TFMPP was inactive. Furthermore, TFMPP increased the release mediated by the serotonin transporter (SERT) in a manner similar to MDMA whereas BZP was inactive. Using microdialysis sampling from rat nucleus accumbens, they also showed that MDMA increase dopamine and serotonin levels. Using BZP, the increase in dopamine dominated compared to the increase in serotonin. TFMPP increased serotonin but had no effect on dopamine. It was also shown that when administered at a 1:1 ratio (3 mg/kg), the increases in dopamine and serotonin levels were comparable to those of low-dose MDMA.

A clinical study (Lin et al. 2011) reported increased pulse and heartbeat, dexamphetamine-like effects, dysphoria, and increased self-confidence after intake of 100 mg BZP and 30 mg TFMPP. With the exception of dysphoria, these effects appear similar to those of MDMA. Thompson et al. (2010) reported improved driving performance (standard deviation of lateral position), agitation, anxiety, hallucinations, vomiting, insomnia, and migraine following a dose of 300 mg BZP and 74 mg TFMPP. Blood concentrations peaked around 600 and 40 ng/mL BZP and TFMPP, respectively.

Nineteen fatalities involving BZP/TFMPP have been reported (Elliott 2011), but it was concluded that the toxicological significance of BZP/TFMPP could not be established due to a wide range of overlapping concentrations.

6.2 Diphenidine and Methoxydiphenidine

Both diphenidine and methoxydiphenidine (MXP) have been indicated to interact with the N-methyl-D-aspartate receptor (NMDA) receptor by binding to membrane preparations from whole rat brain (Gray and Cheng 1989) and, in the case of diphenidine, by reducing NMDA-mediated field excitatory postsynaptic potentials (Wallach et al. 2016). Clinical observations in intoxicated patients showed an altered level of consciousness, hallucinations, confusion, disorientation, and dissociation as well as hypertension, tachycardia, anxiety, and agitation (Helander et al. 2015).

Elliot et al. (2015) reported two cases where MXP was ruled the cause of death (in one case in combination with heart disease). Diphenidine was determined cause of death in combination with other drugs in three cases (Hasegawa et al. 2014b; Kudo et al. 2015; Kusano et al. 2017) and in two of them the stated blood concentrations appeared to be in a similar range as those observed for MXP.

6.3 Methoxetamine

Methoxetamine (MXE) was shown to have an appreciable affinity for the NMDA receptor comparable to ketamine and also showed affinity toward SERT (Roth et al. 2013). Wood et al. (2012) reported a dissociative/catatonic state in combination with tachycardia and hypertension in a patient admitted to hospital after taking MXE. MXE concentrations ranged from 90 to 200 ng/mL in serum ($n = 3$).

Wikström et al. (2013) reported similar concentrations in living users and a concentration of 8,600 ng/mL in a case of fatal MXE intoxication (three synthetic cannabinoids were also present). A similarly high concentration of MXE was reported in another fatality. In that case, the cause of death was asphyxia, but no details were given (EMCDDA 2013b). Miscellaneous drugs and their respective case report data are compiled in Table 4.

Table 4 Summary of the case report data of poisonings with miscellaneous drugs

Substance	CoD	N	Administration	C blood (ng/mL)	Antemortem and postmortem findings	References
1-Benzylpiperazine (BZP) and 1-(3-trifluoromethyl-phenyl) piperazine (TFMPP)	Intoxication[a]	7		BZP ND[b]-1,910	None stated	Elliott (2011)
				TFMPP ND[b]-80		
	Mixed intoxication	6		BZP ND[b]-680		
				TFMPP ND[b]-40		
	Other	6		BZP ND[b]-3,200		
				TFMPP ND[b]-300		
1-Benzylpiperazine (BZP) and 1-(3-trifluoromethyl-phenyl) piperazine (TFMPP)	Mixed intoxication	1		BZP > 250	None stated	Swortwood et al. (2013)
				TFMPP: 93		
Diphenidine	Mixed intoxication	1		1,380	PM: pulmonary congestion and edema	Kudo et al. (2015)
	Mixed intoxication	1		715 (femoral)	None stated	Hasegawa et al. (2014b)
	Mixed intoxication	1		12	None stated	Kusano et al. (2017)
Methoxydiphenidine (MXP)	Intoxication	2[c]		2,000 and 24,000	PM: enlarged heart	Elliott et al. (2015)
	Other	1		1,360		
Methoxetamine (MXE)	Intoxication	1[d]		8,600 ng/g	PM: pulmonary edema	Wikstrom et al. (2013)
	Living	4		130–490 ng/g		
Methoxetamine (MXE)	Mixed intoxication	1	Insufflation	320 (serum)	AM: respiratory and hepatic failure, acute renal insufficiency, hyperthermia, rhabdomyolysis, tachycardia, inflammatory changes of lung, deep coma, seizures, and increased glucose and protein levels	Wiergowski et al. (2014)
					PM: pulmonary, cerebral, and generalized edema, pleural and	

(continued)

Table 4 (continued)

Substance	CoD	N	Administration	C blood (ng/mL)	Antemortem and postmortem findings	References
					peritoneal effusions, parenchymatous lesions in the kidneys, and acute tubular necrosis	
Methoxetamine (MXE)	Mixed intoxication	4		30 and 890 ($n = 2$)[e,f]	None stated	Chiappini et al. (2015)
	Other	3		220 ($n = 1$)[e]		
	Natural	1		Present		
Methoxetamine (MXE)	Other	2[g]		9,480 ($n = 1$)	None stated	EMCDDA (2013b)
Methoxetamine (MXE)	Mixed intoxication	1		64	None stated	Karinen et al. (2014)
Methoxetamine (MXE)	Intoxication	1		5800[h]	None stated	Adamowicz and Zuba (2015)

AM antemortem, *CoD* cause of death, *ND* not detected, *PM* postmortem
[a]Reported as having no alternative CoD
[b]Drug detected in urine
[c]Heart disease included in CoD in one case
[d]Contribution from synthetic cannabinoids cannot be ruled out
[e]The other cases methoxetamine reported as present
[f]1,100 ng/mL in preserved blood
[g]Reporting only cases not covered by other publications and analytically confirmed
[h]Estimated from dried blood on cardboard

7 Conclusions

It is concluded that the most serious threat to drug users stems from synthetic opioids that, given their potent central nervous depressant effects, have an alarming number of accidental deaths spread over the entire globe. The synthetic cannabinoids appeared to be the most unpredictable species with no clear toxidrome and unknown or poorly understood mechanisms of toxicity, but with adverse effects pointing toward the cardiovascular system. The toxidromes commonly encountered after ingestion of cathinones and phenethylamines are of sympathomimetic and hallucinogenic character with a risk of serotonin syndrome, excited delirium, psychosis, and life-threatening cardiovascular effects. In comparison to classic drugs, such as heroin, cannabis, and amphetamine, most NPS appeared to exhibit more severe adverse effects. The deaths attributed to NPS have dramatically increased in the last 2 years. In the opinion of the authors, this might be attributable to a shift from synthetic cannabinoids and cathinones to the more toxic and dangerously potent fentanyl analogs.

References

Adamowicz P (2016) Fatal intoxication with synthetic cannabinoid MDMB-CHMICA. Forensic Sci Int 261:e5–10

Adamowicz P, Tokarczyk B, Stanaszek R, Slopianka M (2013) Fatal mephedrone intoxication – a case report. J Anal Toxicol 37:37–42

Adamowicz P, Zuba D (2015) Fatal intoxication with methoxetamine. J Forensic Sci 60(Suppl 1): S264–S268

Adamowicz P, Zuba D, Byrska B (2014) Fatal intoxication with 3-methyl-N-methylcathinone (3-MMC) and 5-(2-aminopropyl)benzofuran (5-APB). Forensic Sci Int 245:126–132

Adebamiro A, Perazella MA (2012) Recurrent acute kidney injury following bath salts intoxication. Am J Kidney Dis 59:273–275

Alper RH (1990) Hemodynamic and renin responses to (+−)-DOI, a selective 5-HT2 receptor agonist, in conscious rats. Eur J Pharmacol 175:323–332

Andreasen MF, Telving R, Rosendal I, Eg MB, Hasselstrom JB, Andersen LV (2015) A fatal poisoning involving 25C-NBOMe. Forensic Sci Int 251:e1–e8

Angerer V, Franz F, Schwarze B, Moosmann B, Auwarter V (2016) Reply to "sudden cardiac death following use of the synthetic cannabinoid MDMB-CHMICA". J Anal Toxicol 40:240–242

Angerer V, Jacobi S, Franz F, Auwarter V, Pietsch J (2017) Three fatalities associated with the synthetic cannabinoids 5F-ADB, 5F-PB-22, and AB-CHMINACA. Forensic Sci Int 281:e9–e15

Armenian P, Olson A, Anaya A, Kurtz A, Ruegner R, Gerona RR (2017) Fentanyl and a novel synthetic opioid U-47700 masquerading as street "Norco" in Central California: a case report. Ann Emerg Med 69:87–90

Aromatario M, Bottoni E, Santoni M, Ciallella C (2012) New "lethal highs": a case of a deadly cocktail of GHB and mephedrone. Forensic Sci Int 223:e38–e41

Bachs L, Morland H (2001) Acute cardiovascular fatalities following cannabis use. Forensic Sci Int 124:200–203

Bäckberg M, Beck O, Hulten P, Rosengren-Holmberg J, Helander A (2014) Intoxications of the new psychoactive substance 5-(2-aminopropyl)indole (5-IT): a case series from the Swedish STRIDA project. Clin Toxicol (Phila) 52:618–624

Barrios L, Grison-Hernando H, Boels D, Bouquie R, Monteil-Ganiere C, Clement R (2016) Death following ingestion of methylone. Int J Leg Med 130:381–385

Baumann MH, Clark RD, Budzynski AG, Partilla JS, Blough BE, Rothman RB (2005) N-substituted piperazines abused by humans mimic the molecular mechanism of 3,4-methylenedioxymethamphetamine (MDMA, or "Ecstasy"). Neuropsychopharmacology 30:550–560

Behonick G, Shanks KG, Firchau DJ, Mathur G, Lynch CF, Nashelsky M, Jaskierny DJ, Meroueh C (2014) Four postmortem case reports with quantitative detection of the synthetic cannabinoid, 5F-PB-22. J Anal Toxicol 38:559–562

Bersani FS, Corazza O, Albano G, Valeriani G, Santacroce R, Bolzan Mariotti Posocco F, Cinosi E, Simonato P, Martinotti G, Bersani G, Schifano F (2014) 25C-NBOMe: preliminary data on pharmacology, psychoactive effects, and toxicity of a new potent and dangerous hallucinogenic drug. Biomed Res Int 2014:734749

Bishop-Freeman SC, Feaster MS, Beal J, Miller A, Hargrove RL, Brower JO, Winecker RE (2016) Loperamide-related deaths in North Carolina. J Anal Toxicol 40:677–686

Borek HA, Holstege CP (2012) Hyperthermia and multiorgan failure after abuse of "bath salts" containing 3,4-methylenedioxypyrovalerone. Ann Emerg Med 60:103–105

Boulanger-Gobeil C, St-Onge M, Laliberte M, Auger PL (2012) Seizures and hyponatremia related to ethcathinone and methylone poisoning. J Med Toxicol 8:59–61

Brittain RT, Kellett DN, Neat ML, Stables R (1973) Proceedings: anti-nociceptive effects in N-substituted cyclohexylmethylbenzamides. Br J Pharmacol 49:158P–159P

Butler DC, Shanks K, Behonick GS, Smith D, Presnell SE, Tormos LM (2017) Three cases of fatal acrylfentanyl toxicity in the United States and a review of literature. J Anal Toxicol 42:e6–e11

Callaghan PD, Irvine RJ, Daws LC (2005) Differences in the in vivo dynamics of neurotransmitter release and serotonin uptake after acute para-methoxyamphetamine and 3,4-methylenedioxymethamphetamine revealed by chronoamperometry. Neurochem Int 47:350–361

Carbone PN, Carbone DL, Carstairs SD, Luzi SA (2013) Sudden cardiac death associated with methylone use. Am J Forensic Med Pathol 34:26–28

Cawrse BM, Levine B, Jufer RA, Fowler DR, Vorce SP, Dickson AJ, Holler JM (2012) Distribution of methylone in four postmortem cases. J Anal Toxicol 36:434–439

Chiappini S, Claridge H, Corkery JM, Goodair C, Loi B, Schifano F (2015) Methoxetamine-related deaths in the UK: an overview. Hum Psychopharmacol 30:244–248

Coopman V, Blanckaert P, Van Parys G, Van Calenbergh S, Cordonnier J (2016a) A case of acute intoxication due to combined use of fentanyl and 3,4-dichloro-N-[2-(dimethylamino) cyclohexyl]-N-methylbenzamide (U-47700). Forensic Sci Int 266:68–72

Coopman V, Cordonnier J, De Leeuw M, Cirimele V (2016b) Ocfentanil overdose fatality in the recreational drug scene. Forensic Sci Int 266:469–473

Cosbey SH, Peters KL, Quinn A, Bentley A (2013) Mephedrone (methylmethcathinone) in toxicology casework: a Northern Ireland perspective. J Anal Toxicol 37:74–82

Cunningham SM, Haikal NA, Kraner JC (2016) Fatal intoxication with acetyl fentanyl. J Forensic Sci 61(Suppl 1):S276–S280

DailyMed (2014) Naloxone hydrochloride injection, solution. US National Library of Medicine

Daniulaityte R, Juhascik MP, Strayer KE, Sizemore IE, Harshbarger KE, Antonides HM, Carlson RR (2017) Overdose deaths related to fentanyl and its analogs – Ohio, January-February 2017. MMWR Morb Mortal Wkly Rep 66:904–908

Derungs A, Schietzel S, Meyer MR, Maurer HH, Krahenbuhl S, Liechti ME (2011) Sympathomimetic toxicity in a case of analytically confirmed recreational use of naphyrone (naphthylpyrovalerone). Clin Toxicol (Phila) 49:691–693

Dines AM, Wood DM, Galicia M, Yates CM, Heyerdahl F, Hovda KE, Giraudon I, Sedefov R, Euro DENRG, Dargan PI (2015) Presentations to the emergency department following cannabis use – a multi-centre case series from ten European countries. J Med Toxicol 11:415–421

Dziadosz M, Klintschar M, Teske J (2017) Postmortem concentration distribution in fatal cases involving the synthetic opioid U-47700. Int J Leg Med 131(6):1555–1556

ECDD (2014) AH-7921 Critical Review Report Agenda Item 4.21. http://www.who.int/medicines/areas/quality_safety/4_21_review.pdf. 16 Feb 2018
ECDD (2015) MT-45 Critical Review Report Agenda Item 5.1. http://www.who.int/medicines/access/controlled-substances/5.1_MT-45_CRev.pdf. 16 Feb 2018
ECDD (2016) U-47700 Critical Review Report Agenda Item 4.1. http://www.who.int/medicines/access/controlled-substances/4.1_U-47700_CritReview.pdf. 16 Feb 2018
Eggleston W, Clark KH, Marraffa JM (2017) Loperamide abuse associated with cardiac dysrhythmia and death. Ann Emerg Med 69:83–86
Eiden C, Cathala P, Mathieu-Daude JC, Marson B, Baccino E, Leglise Y, Peyriere H (2012) Methadone-related deaths in Montpellier and Region, from 2000 to 2010. Therapie 67:515–522
Ellefsen KN, Taylor EA, Simmons P, Willoughby V, Hall BJ (2017) Multiple drug-toxicity involving novel psychoactive substances, 3-Fluorophenmetrazine and U-47700. J Anal Toxicol:1–6
Elliott S (2011) Current awareness of piperazines: pharmacology and toxicology. Drug Test Anal 3:430–438
Elliott S, Sedefov R, Evans-Brown M (2018) Assessing the toxicological significance of new psychoactive substances in fatalities. Drug Test Anal 10:120–126
Elliott SP, Brandt SD, Smith C (2016) The first reported fatality associated with the synthetic opioid 3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide (U-47700) and implications for forensic analysis. Drug Test Anal 8:875–879
Elliott SP, Brandt SD, Wallach J, Morris H, Kavanagh PV (2015) First reported fatalities associated with the "research chemical" 2-methoxydiphenidine. J Anal Toxicol 39:287–293
EMCDDA-Europol (2010) Joint Report on a new psychoactive substance: 4-methylmethcathinone (mephedrone). http://www.emcdda.europa.eu/attachements.cfm/att_132203_EN_2010_Mephedrone_Joint%20report.pdf. 16 Feb 2018
EMCDDA-Europol (2012) 2011 Annual Report on the implementation of Council Decision 2005/387/JHA. http://www.emcdda.europa.eu/system/files/publications/689/EMCDDA-Europol_Annual_Report_2011_2012_final_335568.pdf. 16 Feb 2018
EMCDDA-Europol (2013) 2012 Annual Report on the implementation of Council Decision 2005/387/JHA (New Drugs in Europe). http://www.emcdda.europa.eu/system/files/publications/734/EMCDDA-Europol_2012_Annual_Report_final_439477.pdf. 16 Feb 2018
EMCDDA–Europol (2009) 2008 Annual Report on the implementation of Council Decision 2005/387/JHA. http://www.emcdda.europa.eu/system/files/publications/550/2008_Implementation_report_281411.pdf. 16 Feb 2018
EMCDDA (2013a) Report on the risk assessment of 5-(2-aminopropyl)indole in the framework of the Council Decision on new psychoactive substances. http://www.emcdda.europa.eu/system/files/publications/788/TDAK13002ENN-1__462975.pdf. 16 Feb 2018
EMCDDA (2013b) Report on the risk assessment of 2-(3-methoxyphenyl)-2-(ethylamino) cyclohexanone (methoxetamine) in the framework of the Council Decision on new psychoactive substances. http://www.emcdda.europa.eu/attachements.cfm/att_228264_EN_TDAK14004ENN.pdf. 16 Feb 2018
EMCDDA (2015) Report on the risk assessment of 1-phenyl-2-(pyrrolidin-1-yl)pentan-1-one (α-pyrrolidinovalerophenone, α-PVP) in the framework of the Council Decision on new psychoactive substances. http://www.emcdda.europa.eu/system/files/publications/2934/TDAK16001ENN.pdf. 16 Feb 2018
Fels H, Krueger J, Sachs H, Musshoff F, Graw M, Roider G, Stoever A (2017) Two fatalities associated with synthetic opioids: AH-7921 and MT-45. Forensic Sci Int 277:e30–e35
Fort C, Curtis B, Nichols C, Niblo C (2016) Acetyl fentanyl toxicity: two case reports. J Anal Toxicol 40:754–757
Garrett G, Sweeney M (2010) The serotonin syndrome as a result of mephedrone toxicity. BMJ Case Rep 2010
Gerace E, Petrarulo M, Bison F, Salomone A, Vincenti M (2014) Toxicological findings in a fatal multidrug intoxication involving mephedrone. Forensic Sci Int 243:68–73

Gieron J, Adamowicz P (2016) Fatal poisoning with the synthetic cannabinoid AB-CHMINACA and ethyl alcohol – a case study and literature review. Problems Forensic Sci 106:482–495

Gil D, Adamowicz P, Skulska A, Tokarczyk B, Stanaszek R (2013) Analysis of 4-MEC in biological and non-biological material – three case reports. Forensic Sci Int 228:e11–e15

Gray NM, Cheng BK (1989) 1,2-diarylethylamines for treatment of neurotoxic injury. SEARLE & CO (US)

Guerrieri D, Rapp E, Roman M, Druid H, Kronstrand R (2017a) Postmortem and toxicological findings in a series of furanylfentanyl-related deaths. J Anal Toxicol 41:242–249

Guerrieri D, Rapp E, Roman M, Thelander G, Kronstrand R (2017b) Acrylfentanyl: another new psychoactive drug with fatal consequences. Forensic Sci Int 277:e21–e29

Halberstadt AL (2017) Pharmacology and toxicology of N-benzylphenethylamine ("NBOMe") hallucinogens. Curr Top Behav Neurosci 32:283–311

Hartung B, Kauferstein S, Ritz-Timme S, Daldrup T (2014) Sudden unexpected death under acute influence of cannabis. Forensic Sci Int 237:e11–e13

Hasegawa K, Suzuki O, Wurita A, Minakata K, Yamagishi I, Nozawa H, Gonmori K, Watanabe K (2014a) Postmortem distribution of α-pyrrolidinovalerophenone and its metabolite in body fluids and solid tissues in a fatal poisoning case measured by LC–MS–MS with the standard addition method. Forensic Toxicol 32:225–234

Hasegawa K, Wurita A, Minakata K, Gonmori K, Nozawa H, Yamagishi I, Watanabe K, Suzuki O (2014b) Postmortem distribution of AB-CHMINACA, 5-fluoro-AMB, and diphenidine in body fluids and solid tissues in a fatal poisoning case: usefulness of adipose tissue for detection of the drugs in unchanged forms. Forensic Toxicol 33:45–53

Hasegawa K, Wurita A, Minakata K, Gonmori K, Nozawa H, Yamagishi I, Watanabe K, Suzuki O (2015) Postmortem distribution of AB-CHMINACA, 5-fluoro-AMB, and diphenidine in body fluids and solid tissues in a fatal poisoning case: usefulness of adipose tissue for detection of the drugs in unchanged forms. Forensic Toxicol 33:45–53

Hayes AG, Tyers MB (1983) Determination of receptors that mediate opiate side effects in the mouse. Br J Pharmacol 79:731–736

Helander A, Backberg M, Signell P, Beck O (2017) Intoxications involving acrylfentanyl and other novel designer fentanyls – results from the Swedish STRIDA project. Clin Toxicol (Phila) 55:589–599

Helander A, Beck O, Backberg M (2015) Intoxications by the dissociative new psychoactive substances diphenidine and methoxphenidine. Clin Toxicol (Phila) 53:446–453

Helland A, Brede WR, Michelsen LS, Gundersen POM, Aarset H, Skjolas JE, Slordal L (2017) Two hospitalizations and one death after exposure to ortho-fluorofentanyl. J Anal Toxicol 41:708–709

Hess C, Stockhausen S, Kernbach-Wighton G, Madea B (2015) Death due to diabetic ketoacidosis: induction by the consumption of synthetic cannabinoids? Forensic Sci Int 257:e6–11

Johansen SS, Hansen AC, Muller IB, Lundemose JB, Franzmann MB (2003) Three fatal cases of PMA and PMMA poisoning in Denmark. J Anal Toxicol 27:253–256

Karinen R, Tuv SS, Rogde S, Peres MD, Johansen U, Frost J, Vindenes V, Oiestad AM (2014) Lethal poisonings with AH-7921 in combination with other substances. Forensic Sci Int 244: e21–e24

Kasper AM, Ridpath AD, Arnold JK, Chatham-Stephens K, Morrison M, Olayinka O, Parker C, Galli R, Cox R, Preacely N, Anderson J, Kyle PB, Gerona R, Martin C, Schier J, Wolkin A, Dobbs T (2015) Severe illness associated with reported use of synthetic cannabinoids – Mississippi, April 2015. MMWR Morb Mortal Wkly Rep 64:1121–1122

Katz KD, Leonetti AL, Bailey BC, Surmaitis RM, Eustice ER, Kacinko S, Wheatley SM (2016) Case series of synthetic cannabinoid intoxication from one toxicology center. West J Emerg Med 17:290–294

Kesha K, Boggs CL, Ripple MG, Allan CH, Levine B, Jufer-Phipps R, Doyon S, Chi P, Fowler DR (2013) Methylenedioxypyrovalerone ("bath salts"), related death: case report and review of the literature. J Forensic Sci 58:1654–1659

Khademi H, Kamangar F, Brennan P, Malekzadeh R (2016) Opioid therapy and its side effects: a review. Arch Iran Med 19:870–876

Kronstrand R, Roman M, Andersson M, Eklund A (2013a) Toxicological findings of synthetic cannabinoids in recreational users. J Anal Toxicol 37:534–541

Kronstrand R, Roman M, Dahlgren M, Thelander G, Wikstrom M, Druid H (2013b) A cluster of deaths involving 5-(2-aminopropyl)indole (5-IT). J Anal Toxicol 37:542–546

Kronstrand R, Thelander G, Lindstedt D, Roman M, Kugelberg FC (2014) Fatal intoxications associated with the designer opioid AH-7921. J Anal Toxicol 38:599–604

Krotulski AJ, Papsun DM, Friscia M, Swartz JL, Holsey BD, Logan BK (2017) Fatality following ingestion of tetrahydrofuranylfentanyl, U-49900 and methoxy-phencyclidine. J Anal Toxicol:1–6

Kudo K, Usumoto Y, Kikura-Hanajiri R, Sameshima N, Tsuji A, Ikeda N (2015) A fatal case of poisoning related to new cathinone designer drugs, 4-methoxy PV8, PV9, and 4-methoxy PV9, and a dissociative agent, diphenidine. Leg Med (Tokyo) 17:421–426

Kueppers VB, Cooke CT (2015) 25I-NBOMe related death in Australia: a case report. Forensic Sci Int 249:e15–e18

Kusano M, Zaitsu K, Taki K, Hisatsune K, Nakajima J, Moriyasu T, Asano T, Hayashi Y, Tsuchihashi H, Ishii A (2017) Fatal intoxication by 5F-ADB and diphenidine: detection, quantification, and investigation of their main metabolic pathways in humans by LC/MS/MS and LC/Q-TOFMS. Drug Test Anal 10:1–10

Lasoff DR, Koh CH, Corbett B, Minns AB, Cantrell FL (2017) Loperamide trends in abuse and misuse over 13 years: 2002-2015. Pharmacotherapy 37:249–253

Lin DL, Liu HC, Yin HL (2007) Recent paramethoxymethamphetamine (PMMA) deaths in Taiwan. J Anal Toxicol 31:109–113

Lin JC, Jan RK, Lee H, Jensen MA, Kydd RR, Russell BR (2011) Determining the subjective and physiological effects of BZP combined with TFMPP in human males. Psychopharmacology (Berl) 214:761–768

Loi B, Corkery JM, Claridge H, Goodair C, Chiappini S, Gimeno Clemente C, Schifano F (2015) Deaths of individuals aged 16-24 years in the UK after using mephedrone. Hum Psychopharmacol 30:225–232

Lowe LM, Peterson BL, Couper FJ (2015) A case review of the first analytically confirmed 25I-NBOMe-related death in Washington state. J Anal Toxicol 39:668–671

Luciano RL, Perazella MA (2014) Nephrotoxic effects of designer drugs: synthetic is not better! Nat Rev Nephrol 10:314–324

Lurie Y, Gopher A, Lavon O, Almog S, Sulimani L, Bentur Y (2012) Severe paramethoxymethamphetamine (PMMA) and paramethoxyamphetamine (PMA) outbreak in Israel. Clin Toxicol (Phila) 50:39–43

Lusthof KJ, Oosting R, Maes A, Verschraagen M, Dijkhuizen A, Sprong AG (2011) A case of extreme agitation and death after the use of mephedrone in the Netherlands. Forensic Sci Int 206:e93–e95

Maeda H, Kikura-Hanajiri R, Kawamura M, Nagashima E, Yoshida KI (2017) AB-CHMINACA-induced sudden death from non-cardiogenic pulmonary edema. Clin Toxicol (Phila) 56:1–3

Martin TL (2001) Three cases of fatal paramethoxyamphetamine overdose. J Anal Toxicol 25:649–651

Martucci HFH, Ingle EA, Hunter MD, Rodda LN (2018) Distribution of furanyl fentanyl and 4-ANPP in an accidental acute death: a case report. Forensic Sci Int 283:e13–e17

Mash DC, Duque L, Pablo J, Qin Y, Adi N, Hearn WL, Hyma BA, Karch SB, Druid H, Wetli CV (2009) Brain biomarkers for identifying excited delirium as a cause of sudden death. Forensic Sci Int 190:e13–e19

Maskell PD, De Paoli G, Seneviratne C, Pounder DJ (2011) Mephedrone (4-methylmethcathinone)-related deaths. J Anal Toxicol 35:188–191

McIntyre IM, Gary RD, Joseph S, Stabley R (2017) A fatality related to the synthetic opioid U-47700: postmortem concentration distribution. J Anal Toxicol 41:158–160

McIntyre IM, Gary RD, Trochta A, Stolberg S, Stabley R (2015a) Acute 5-(2-aminopropyl) benzofuran (5-APB) intoxication and fatality: a case report with postmortem concentrations. J Anal Toxicol 39:156–159
McIntyre IM, Hamm CE, Aldridge L, Nelson CL (2013) Acute methylone intoxication in an accidental drowning – a case report. Forensic Sci Int 231:e1–e3
McIntyre IM, Trochta A, Gary RD, Malamatos M, Lucas JR (2015b) An acute acetyl fentanyl fatality: a case report with postmortem concentrations. J Anal Toxicol 39:490–494
McIntyre IM, Trochta A, Gary RD, Wright J, Mena O (2016) An acute butyr-fentanyl fatality: a case report with postmortem concentrations. J Anal Toxicol 40:162–166
Mercer SL, Coop A (2011) Opioid analgesics and P-glycoprotein efflux transporters: a potential systems-level contribution to analgesic tolerance. Curr Top Med Chem 11:1157–1164
Mofenson HC, Greensher J (1970) The nontoxic ingestion. Pediatr Clin North Am 17:583–590
Mohr AL, Friscia M, Papsun D, Kacinko SL, Buzby D, Logan BK (2016) Analysis of novel synthetic opioids U-47700, U-50488 and furanyl fentanyl by LC-MS/MS in postmortem casework. J Anal Toxicol 40:709–717
Mounteney J, Giraudon I, Denissov G, Griffiths P (2015) Fentanyls: are we missing the signs? Highly potent and on the rise in Europe. Int J Drug Policy 26:626–631
Murray BL, Murphy CM, Beuhler MC (2012) Death following recreational use of designer drug "bath salts" containing 3,4-Methylenedioxypyrovalerone (MDPV). J Med Toxicol 8:69–75
Nagai H, Saka K, Nakajima M, Maeda H, Kuroda R, Igarashi A, Tsujimura-Ito T, Nara A, Komori M, Yoshida K (2014) Sudden death after sustained restraint following self-administration of the designer drug alpha-pyrrolidinovalerophenone. Int J Cardiol 172:263–265
Nakamura H, Shimizu M (1976) 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 221:105–121
O'Donnell JK, Halpin J, Mattson CL, Goldberger BA, Gladden RM (2017) Deaths involving fentanyl, fentanyl analogs, and U-47700 – 10 states, July-December 2016. MMWR Morb Mortal Wkly Rep 66:1197–1202
Patton AL, Chimalakonda KC, Moran CL, McCain KR, Radominska-Pandya A, James LP, Kokes C, Moran JH (2013) K2 toxicity: fatal case of psychiatric complications following AM2201 exposure. J Forensic Sci 58:1676–1680
Paul ABM, Simms L, Amini S, Paul AE (2017) Teens and spice: a review of adolescent fatalities associated with synthetic cannabinoid use. J Forensic Sci. https://doi.org/10.1111/1556-4029.13704
Pearson JM, Hargraves TL, Hair LS, Massucci CJ, Frazee CC 3rd, Garg U, Pietak BR (2012) Three fatal intoxications due to methylone. J Anal Toxicol 36:444–451
Penders TM (2013) The syndrome of excited delirium following use of "bath salts". J Clin Psychiatry 74:518
Penders TM, Gestring R (2011) Hallucinatory delirium following use of MDPV: "Bath Salts". Gen Hosp Psychiatry 33:525–526
Penders TM, Gestring RE (2014) Letter to the editor – methylenedioxypyrovalerone as a cause of excited delirium. J Forensic Sci 59:869
Penders TM, Gestring RE, Vilensky DA (2012a) Excited delirium following use of synthetic cathinones (bath salts). Gen Hosp Psychiatry 34:647–650
Penders TM, Gestring RE, Vilensky DA (2012b) Intoxication delirium following use of synthetic cathinone derivatives. Am J Drug Alcohol Abuse 38:616–617
Pereira J, Lawlor P, Vigano A, Dorgan M, Bruera E (2001) Equianalgesic dose ratios for opioids. A critical review and proposals for long-term dosing. J Pain Symptom Manage 22:672–687
Poklis J, Poklis A, Wolf C, Hathaway C, Arbefeville E, Chrostowski L, Devers K, Hair L, Mainland M, Merves M, Pearson J (2016) Two fatal intoxications involving butyryl fentanyl. J Anal Toxicol 40:703–708
Poklis J, Poklis A, Wolf C, Mainland M, Hair L, Devers K, Chrostowski L, Arbefeville E, Merves M, Pearson J (2015) Postmortem tissue distribution of acetyl fentanyl, fentanyl and

their respective nor-metabolites analyzed by ultrahigh performance liquid chromatography with tandem mass spectrometry. Forensic Sci Int 257:435–441

Poklis JL, Devers KG, Arbefeville EF, Pearson JM, Houston E, Poklis A (2014) Postmortem detection of 25I-NBOMe [2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl] ethanamine] in fluids and tissues determined by high performance liquid chromatography with tandem mass spectrometry from a traumatic death. Forensic Sci Int 234:e14–e20

Potocka-Banas B, Janus T, Majdanik S, Banas T, Dembinska T, Borowiak K (2017) Fatal intoxication with alpha-PVP, a synthetic cathinone derivative. J Forensic Sci 62:553–556

Rickli A, Luethi D, Reinisch J, Buchy D, Hoener MC, Liechti ME (2015) Receptor interaction profiles of novel N-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). Neuropharmacology 99:546–553

Rohrig TP, Miller SA, Baird TR (2017) U-47700: a not so new opioid. J Anal Toxicol 42:e12–e14

Roth BL, Gibbons S, Arunotayanun W, Huang XP, Setola V, Treble R, Iversen L (2013) The ketamine analogue methoxetamine and 3- and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor. PLoS One 8:e59334

Saito T, Namera A, Miura N, Ohta S, Miyazaki S, Osawa M, Inokuchi S (2013) A fatal case of MAM-2201 poisoning. Forensic Toxicol 31:333–337

Sasaki C, Saito T, Shinozuka T, Irie W, Murakami C, Maeda K, Nakamaru N, Oishi M, Nakamura S, Kurihara K (2015) A case of death caused by abuse of a synthetic cannabinoid N-1-naphthalenyl-1-pentyl-1H-indole-3-carboxamide. Forensic Toxicol 33:165–169

Schaefer N, Peters B, Bregel D, Kneisel S, Auwaerter V, Schmidt PH, Ewald AH (2013) A fatal case involving several synthetic cannabinoids. Toxichem Krimtech:248–251

Seetohul LN, Pounder DJ (2013) Four fatalities involving 5-IT. J Anal Toxicol 37:447–451

Shanks KG, Behonick GS (2016) Death after use of the synthetic cannabinoid 5F-AMB. Forensic Sci Int 262:e21–e24

Shanks KG, Behonick GS, Archuleta PA, Jaskierny DJ (2014) Case reports: fatalities associated with the synthetic cannabinoid AB-PINACA. In: Society of Forensic Toxicologists (SOFT) annual meeting abstract proceedings, Grand Rapids

Shanks KG, Clark W, Behonick G (2016) Death associated with the use of the synthetic cannabinoid ADB-FUBINACA. J Anal Toxicol 40:236–239

Shanks KG, Dahn T, Terrell AR (2012) Detection of JWH-018 and JWH-073 by UPLC-MS-MS in postmortem whole blood casework. J Anal Toxicol 36:145–152

Shanks KG, Sozio T, Behonick GS (2015a) Fatal intoxications with 25B-NBOMe and 25I-NBOMe in Indiana during 2014. J Anal Toxicol 39:602–606

Shanks KG, Winston D, Heidingsfelder J, Behonick G (2015b) Case reports of synthetic cannabinoid XLR-11 associated fatalities. Forensic Sci Int 252:e6–e9

Shoff EN, Zaney ME, Kahl JH, Hime GW, Boland DM (2017) Qualitative identification of fentanyl analogs and other opioids in postmortem cases by UHPLC-ion trap-MSn. J Anal Toxicol 41:484–492

Simmler LD, Rickli A, Hoener MC, Liechti ME (2014) Monoamine transporter and receptor interaction profiles of a new series of designer cathinones. Neuropharmacology 79:152–160

Spiller HA, Ryan ML, Weston RG, Jansen J (2011) Clinical experience with and analytical confirmation of "bath salts" and "legal highs" (synthetic cathinones) in the United States. Clin Toxicol (Phila) 49:499–505

Swanson DM, Hair LS, Strauch Rivers SR, Smyth BC, Brogan SC, Ventoso AD, Vaccaro SL, Pearson JM (2017) Fatalities involving carfentanil and furanyl fentanyl: two case reports. J Anal Toxicol 41:498–502

Swortwood MJ, Boland DM, DeCaprio AP (2013) Determination of 32 cathinone derivatives and other designer drugs in serum by comprehensive LC-QQQ-MS/MS analysis. Anal Bioanal Chem 405:1383–1397

Sykutera M, Cychowska M, Bloch-Boguslawska E (2015) A fatal case of pentedrone and alpha-pyrrolidinovalerophenone poisoning. J Anal Toxicol 39:324–329

Szmuszkovicz J, Von Voigtlander PF (1982) Benzeneacetamide amines: structurally novel non-m mu opioids. J Med Chem 25:1125–1126
Tait RJ, Caldicott D, Mountain D, Hill SL, Lenton S (2016) A systematic review of adverse events arising from the use of synthetic cannabinoids and their associated treatment. Clin Toxicol 54:1–13
Takase I, Koizumi T, Fujimoto I, Yanai A, Fujimiya T (2016) An autopsy case of acetyl fentanyl intoxication caused by insufflation of "designer drugs". Leg Med (Tokyo) 21:38–44
Tarpgaard M, Maerkedahl R, Lauridsen KB (2015) Fatal intoxication with the new designer drug 25C-NBOMe. Ugeskr Laeger 177(35). pii: V09140523
Thirakul P, L SH KLB, MP J (2017) Clinical presentation, autopsy results and toxicology findings in an acute N-ethylpentylone fatality. J Anal Toxicol 41:342–346
Thompson I, Williams G, Caldwell B, Aldington S, Dickson S, Lucas N, McDowall J, Weatherall M, Robinson G, Beasley R (2010) Randomised double-blind, placebo-controlled trial of the effects of the "party pills" BZP/TFMPP alone and in combination with alcohol. J Psychopharmacol 24:1299–1308
Thornton SL, Gerona RR, Tomaszewski CA (2012) Psychosis from a bath salt product containing flephedrone and MDPV with serum, urine, and product quantification. J Med Toxicol 8:310–313
Tiscione NB, Shanks KG (2016) Carfentanil identified in two driving under the influence of drugs cases. Toxtalk 40:14–17
Torrance H, Cooper G (2010) The detection of mephedrone (4-methylmethcathinone) in 4 fatalities in Scotland. Forensic Sci Int 202:e62–e63
Trecki J, Gerona RR, Schwartz MD (2015) Synthetic cannabinoid-related illnesses and deaths. N Engl J Med 373:103–107
UNODC (2017a) Global overview of drug demand and supply. World Drug Report 2017
UNODC (2017b) Market analysis of synthetic drugs. World Drug Report 2017
Usui K, Aramaki T, Hashiyada M, Hayashizaki Y, Funayama M (2014) Quantitative analysis of 3,4-dimethylmethcathinone in blood and urine by liquid chromatography-tandem mass spectrometry in a fatal case. Leg Med (Tokyo) 16:222–226
Usui K, Fujita Y, Kamijo Y, Kokaji T, Funayama M (2017) Identification of 5-fluoro ADB in human whole blood in four death cases. J Anal Toxicol 42:e21–e25
Vakkalanka JP, Charlton NP, Holstege CP (2017) Epidemiologic trends in loperamide abuse and misuse. Ann Emerg Med 69:73–78
Wallach J, Kang H, Colestock T, Morris H, Bortolotto ZA, Collingridge GL, Lodge D, Halberstadt AL, Brandt SD, Adejare A (2016) Pharmacological investigations of the dissociative "Legal Highs" diphenidine, methoxphenidine and analogues. PLoS One 11:e0157021
Walterscheid JP, Phillips GT, Lopez AE, Gonsoulin ML, Chen HH, Sanchez LA (2014) Pathological findings in 2 cases of fatal 25I-NBOMe toxicity. Am J Forensic Med Pathol 35:20–25
Warrick BJ, Wilson J, Hedge M, Freeman S, Leonard K, Aaron C (2012) Lethal serotonin syndrome after methylone and butylone ingestion. J Med Toxicol 8:65–68
Westin AA, Frost J, Brede WR, Gundersen POM, Einvik S, Aarset H, Slørdal L (2016) Sudden cardiac death following use of the synthetic cannabinoid MDMB-CHMICA. J Anal Toxicol 40:86–87
Westphal F, Junge T, Rosner P, Sonnichsen F, Schuster F (2009) Mass and NMR spectroscopic characterization of 3,4-methylenedioxypyrovalerone: a designer drug with alpha-pyrrolidinophenone structure. Forensic Sci Int 190:1–8
Vevelstad M, Oiestad EL, Middelkoop G, Hasvold I, Lilleng P, Delaveris GJ, Eggen T, Morland J, Arnestad M (2012) The PMMA epidemic in Norway: comparison of fatal and non-fatal intoxications. Forensic Sci Int 219:151–157
White JM, Irvine RJ (1999) Mechanisms of fatal opioid overdose. Addiction 94:961–972
Wiergowski M, Anand JS, Krzyzanowski M, Jankowski Z (2014) Acute methoxetamine and amphetamine poisoning with fatal outcome: a case report. Int J Occup Med Environ Health 27:683–690

Wikstrom M, Thelander G, Dahlgren M, Kronstrand R (2013) An accidental fatal intoxication with methoxetamine. J Anal Toxicol 37:43–46

Wikstrom M, Thelander G, Nystrom I, Kronstrand R (2010) Two fatal intoxications with the new designer drug methedrone (4-methoxymethcathinone). J Anal Toxicol 34:594–598

Winklhofer S, Surer E, Ampanozi G, Ruder T, Stolzmann P, Elliott M, Oestreich A, Kraemer T, Thali M, Alkadhi H, Schweitzer W (2014) Post-mortem whole body computed tomography of opioid (heroin and methadone) fatalities: frequent findings and comparison to autopsy. Eur Radiol 24:1276–1282

Winstock A, Mitcheson L, Ramsey J, Davies S, Puchnarewicz M, Marsden J (2011) Mephedrone: use, subjective effects and health risks. Addiction 106:1991–1996

Wood DM, Davies S, Puchnarewicz M, Button J, Archer R, Ovaska H, Ramsey J, Lee T, Holt DW, Dargan PI (2010) Recreational use of mephedrone (4-methylmethcathinone, 4-MMC) with associated sympathomimetic toxicity. J Med Toxicol 6:327–330

Wood DM, Davies S, Puchnarewicz M, Johnston A, Dargan PI (2012) Acute toxicity associated with the recreational use of the ketamine derivative methoxetamine. Eur J Clin Pharmacol 68:853–856

Wood DM, Greene SL, Dargan PI (2011) Clinical pattern of toxicity associated with the novel synthetic cathinone mephedrone. Emerg Med J 28:280–282

Wood DM, Greene SL, Dargan PI (2013) Emergency department presentations in determining the effectiveness of drug control in the United Kingdom: mephedrone (4-methylmethcathinone) control appears to be effective using this model. Emerg Med J 30:70–71

Vorce SP, Knittel JL, Holler JM, Magluilo J Jr, Levine B, Berran P, Bosy TZ (2014) A fatality involving AH-7921. J Anal Toxicol 38:226–230

Wright TH, Cline-Parhamovich K, Lajoie D, Parsons L, Dunn M, Ferslew KE (2013) Deaths involving methylenedioxypyrovalerone (MDPV) in upper East Tennessee. J Forensic Sci 58:1558–1562

Wurita A, Hasegawa K, Minakata K, Gonmori K, Nozawa H, Yamagishi I, Suzuki O, Watanabe K (2016) Identification and quantification of metabolites of AB-CHMINACA in a urine specimen of an abuser. Leg Med (Tokyo) 19:113–118

Wyman JF, Lavins ES, Engelhart D, Armstrong EJ, Snell KD, Boggs PD, Taylor SM, Norris RN, Miller FP (2013) Postmortem tissue distribution of MDPV following lethal intoxication by "bath salts". J Anal Toxicol 37:182–185

Yeter O (2017) Identification of the synthetic cannabinoid 1-(4-cyanobutyl)-N-(2-phenylpropan-2-yl)-1H-indazole-3-carboxamide (CUMYL-4CN-BINACA) in plant material and quantification in post-mortem blood samples. J Anal Toxicol 41:720–728

Yonemitsu K, Sasao A, Mishima S, Ohtsu Y, Nishitani Y (2016) A fatal poisoning case by intravenous injection of "bath salts" containing acetyl fentanyl and 4-methoxy PV8. Forensic Sci Int 267:e6–e9

Zaitsu K, Nakayama H, Yamanaka M, Hisatsune K, Taki K, Asano T, Kamata T, Katagai M, Hayashi Y, Kusano M, Tsuchihashi H, Ishii A (2015) High-resolution mass spectrometric determination of the synthetic cannabinoids MAM-2201, AM-2201, AM-2232, and their metabolites in postmortem plasma and urine by LC/Q-TOFMS. Int J Leg Med 129:1233–1245

Zuba D, Sekula K, Buczek A (2013) 25C-NBOMe – new potent hallucinogenic substance identified on the drug market. Forensic Sci Int 227:7–14

Wastewater Analysis for Community-Wide Drugs Use Assessment

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Contents

Abstract

Wastewater-based epidemiology (WBE) complements existing epidemiology-based estimation techniques and provides objective, evidence-based estimates of illicit drug use. After consumption, biomarkers – drugs and their metabolites – excreted to toilets and flushed into urban sewer networks can be measured in raw wastewater samples. The quantified loads can serve as an estimate for the collective consumption of all people contributing to the wastewater sample. This transdisciplinary approach, further explained in this chapter, has developed, matured and is now established for monitoring substances such as cocaine and amphetamine-type stimulants. Research currently underway is refining WBE to new applications including new psychoactive substances (NPS).

Keywords

Illicit drugs · New psychoactive substances · Public health · Wastewater-based epidemiology

1 From Sewage to Drug Consumption: The Concept of Wastewater-Based Epidemiology

Over the last decade, wastewater-based epidemiology (WBE) has been developed as a complementary approach to obtain an objective estimate for the collective drug consumption of an entire population, typically at a city level (EMCDDA 2016; Ort et al. 2014a). Along with other data such as information on drug-related criminality, hospital records, and population surveys, WBE allows a better understanding of drug use and gives policymakers a wealth of information useful to take evidence-based decisions and assess the impact of prevention strategies and enforcement actions (Been et al. 2016b, c; Thomaidis et al. 2016; Zuccato et al. 2016). WBE has demonstrated its value and is well established for monitoring conventional illicit drugs such as cocaine, MDMA, amphetamine, and methamphetamine (EMCDDA 2016). It also has

potential to monitor trends in new psychoactive substance (NPS) use following the same workflow used for "classical" drugs. In this chapter, the principles of WBE, strengths and challenges for conventional drugs are explained first. Furthermore, the focus of Sect. 5 is on the current status and potential of WBE for monitoring NPS.

WBE relies on the fact that human metabolic excretion products resulting from substance use are collected and pooled by central sewage systems. Therefore, the measurement of target metabolic residues in untreated urban wastewater allows for identifying the use of specific substances, providing valuable data on the amount and types of substances consumed by the population contributing to the sampled wastewater (van Nuijs et al. 2011a). Hence, consumption can be estimated following the subsequent steps (Gracia-Lor et al. 2017a; Zuccato et al. 2008; see also scheme in Fig. 1):

1. Collection of a representative composite sample – typically over 24 h – of influent wastewater (see Sect. 3.1). Result: a small, pooled wastewater sample (100 mL–1 L) resembling the properties of the entire wastewater volume entering a wastewater treatment plant (WWTP), normally several thousand cubic meters of wastewater per day
2. Chemical analyses for selected biomarkers (see Sect. 3.2). Result: average concentration of biomarker (ng/L) over the sampled period of time in the entire wastewater volume
3. Multiplication of average concentrations (ng/L) with measured flow rates of wastewater (m^3/day). Result: Biomarker mass load in the wastewater (mg/day)
4. Normalization of biomarker mass load by the number of people, who contributed to the sample, to facilitate comparison among cities of different sizes. Result: population-normalized biomarker load in the wastewater (mg/day/1,000 people)
5. Application of a specific correction factor (CF), which considers the average excretion rate of a given drug residue and the molecular mass ratio of parent drug/metabolite. Result: estimation of total consumption of a substance (mg/day/1,000 people)

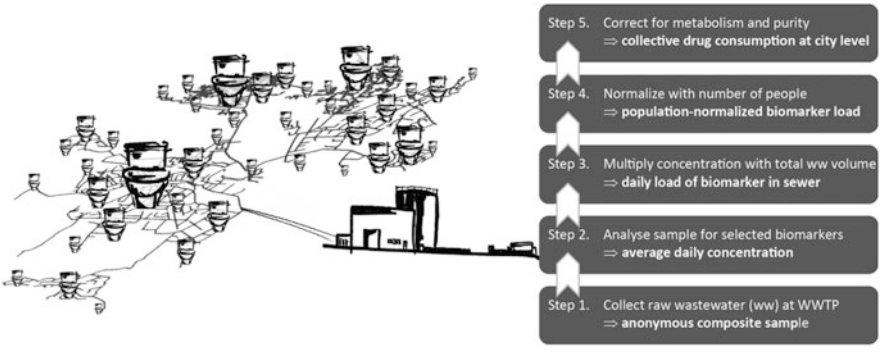

Fig. 1 Key steps in estimating illicit drug use at the community level based on urban wastewater collected at the influent of a wastewater treatment plant (WWTP) (modified from Thomas et al. 2012)

Although this concept is relatively simple, various factors influencing its reliability need to be fully understood before implementation on a large scale is possible (Castiglioni et al. 2013). Among the most important factors are human metabolic and excretion patterns of the investigated drugs, characteristics of sewer systems (the number of people contributing to a sample, wastewater volumes), and understanding the fate of biomarkers in the sewer system (e.g., stability, degradation, partitioning, or sorption in the sewer [McCall et al. 2016; Ramin et al. 2017]).

The WBE approach can be designed to study substance use in a specific area or to compare the use among different areas during defined periods of the year or over successive years (Ort et al. 2014a). As a result, it has the potential to provide almost real-time information on drug use patterns, both geographical and temporal, including during special events or holidays.

However, there are a few limitations of the approach, as WBE cannot provide information on prevalence and frequency of use, route of administration, main classes of users, and purity of substances. Furthermore, translating the total consumed amounts into the corresponding number of average doses is complicated as amounts of drugs taken vary widely, and purity levels fluctuate (EMCDDA 2016). WBE is therefore proposed to complement, rather than to replace established socio-epidemiological monitoring tools (Been et al. 2016a; EMCDDA 2016).

2 Choice of Biomarkers

Specific metabolic excretion residues (biomarkers) that enter urban wastewater may either be the parent substance or a metabolite. Metabolites are preferable since the presence of the parent compound may also originate from disposal/dumping of the unused drug into the sewer system. In cases where unique metabolic products are lacking, the presence of dumping can be addressed by chiral analysis and their characteristic stereoselective metabolism patterns if the drug of question contains asymmetric centers (see Sect. 3.2). The selection of a specific biomarker is not an easy task, because it should fulfill several requirements to ensure the reliability of back-calculated estimates. An ideal biomarker should be excreted in consistent amounts in urine, be detectable in urban wastewater, be stable in wastewater, and be released into sewers only as a result of human excretion (EMCDDA 2016). Therefore, all these characteristics should be verified carefully before a substance – parent or metabolite – can be used as a biomarker.

It is particularly relevant to assess the stability of a biomarker in wastewater, during transport in the sewer, sampling, and analysis. The degradation of a substance can easily occur in wastewater as a result of the high microbial activity typically found in sewers. For instance, it was shown that metabolites excreted as glucuronide conjugates (i.e., morphine 3β-glucuronide) are completely reverted into the free form in raw wastewater (Castiglioni et al. 2006; D'Ascenzo et al. 2003). An overview of the stability studies of the most common biomarkers was provided recently (EMCDDA 2016; McCall et al. 2016). It indicated that benzoylecgonine, the main metabolite of cocaine, and the parent substances, amphetamine, methamphetamine,

and MDMA are the most stable biomarkers for these illicit drugs. On the contrary, the specific metabolite of heroin, 6-monoacetylmorphine, was found to be highly unstable in wastewater, thus morphine appears to be so far the best option to estimate heroin consumption, although morphine therapeutic use should be taken into account. This requires a particular care to collect the most accurate figure of morphine use from prescriptions and sales reports, collected at local level if possible. Regarding cannabis, the main metabolite 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) was stable under relevant conditions (EMCDDA 2016). However, other important knowledge gaps as well as analytical challenges related to the determination of THC-COOH have been identified. A study focused on the initial aspects of the analytical procedure suggested a best practice protocol to handle samples in order to improve estimations of cannabis use (Causanilles et al. 2017). Recently, a new modeling framework to assess in-sewer stability was proposed taking into account realistic biotransformation conditions occurring in the sewer system (McCall et al. 2017). This approach, assessing the effect of instability in a specific catchment, is applicable to all substances and includes varying transformation potential of different biofilms in different pipes.

Once the specificity of a biomarker as indicator of human excretion is verified, it is possible to develop a correction factor (CF) to back-calculate the consumption of the parent substance. A CF takes into account the biomarker excretion profile and the molecular mass ratio of the parent drug to the metabolite (in the case the biomarker is a metabolite) (Zuccato et al. 2008). Due to the scant information on pharmacokinetic profiles of illicit drugs and the high variability of results, a comprehensive meta-analysis of the metabolic studies available was conducted in order to develop CFs as much reliable as possible. This was suggested first for cocaine (Castiglioni et al. 2013), and later implemented for the other main illicit drugs (Gracia-Lor et al. 2016). The procedure took into account not only the excretion profile of a drug but also the number of subjects involved in each study, and the frequency of use for each route of administration. This was necessary because different CFs were employed to estimate the use of the same substance. For instance, for cocaine the CFs ranged between 2.3 and 3.2 considering different excretion profiles from single studies. The meta-analysis study facilitated the integration of all available information and proposed a new corrected CF of 3.59 (Castiglioni et al. 2013). Nowadays, it is highly recommended to use reliable and homogeneous CFs to back-calculate illicit drugs use in different studies, ensuring a higher comparability of results from different research groups.

3 Best Practice Protocol

3.1 Sample Collection and Monitoring Schemes

WBE typically relies on routinely collected 24 h composite samples representing the wastewater from an entire day (daily average concentration). Typically, the sampling error for samples from large WWTPs, analyzed for high prevalence drugs, is usually

negligible compared to the overall uncertainty of WBE results (Castiglioni et al. 2013). However, in small WWTPs (e.g., Ort et al. 2014b) or effluents of individual premises – such as prisons or schools (e.g., Brewer et al. 2016; Burgard et al. 2013; Postigo et al. 2011) – and for low prevalence drugs analyzed in larger WWTPs, routine sampling may result in considerable sampling error (Ort et al. 2010c): When the toilet is flushed, the flush initially lasts a few seconds. In the sewer system, depending on layout, topography, and properties of pipes, the flush extends over time (effect of dispersion, Rieckermann et al. 2005) and may last several minutes at the influent of a WWTP (Ort and Gujer 2006; Ort et al. 2010b). If this flush was the only one containing the substance of interest, it would be challenging to properly capture this peak and obtain the true daily average concentration. With an increasing number of toilet flushes containing the same substance arriving at the WWTP, the temporal concentration pattern becomes less variable and, hence, it is less challenging to obtain a representative 24-h composite sample.

Besides the rather random, short-term variations described above, there can also be systematic diurnal variation (e.g., Brewer et al. 2012; Lai et al. 2013). To properly account for both types of variation, it is recommended to rely on a volume-proportional sampling mode with high temporal resolution. More examples and further details can be found in Ort (2014).

Most studies have to date investigated relatively short periods of consecutive days at different intervals, e.g., 1 week annually (e.g., Ort et al. 2014a) or selected months (e.g., Harman et al. 2011; Zuccato et al. 2011). There are, however, some data available from studies carried out at higher frequency over longer periods of time, i.e., daily over several months to years (e.g., Lai et al. 2015; Ort et al. 2014b; van Nuijs et al. 2011b). Such data are useful for studying long-term trends, or indeed seasonal variability of drug use. Ort et al. (2014b) have proposed a stratified random monitoring scheme as the most appropriate for this task. One implementation of "*stratified random*" is, for example, considering each weekday randomly once per quarter, resulting in 28 samples per year. The underlying day-to-day and seasonal variability is ideally quantified with a preliminary high-frequency monitoring campaign or, alternatively, with expert knowledge considering other evidence. Since wastewater samples are usually analyzed for multiple substances, the substance with the highest variability expected is decisive for the minimum number of monitoring days required to achieve a certain accuracy, e.g., to estimate an annual average. Monitoring over longer periods and with samples collected on nonconsecutive days does increase the time and effort required to coordinate the exercise, both from the perspective of the wastewater scientist and the wastewater treatment staff. Deciding on the most appropriate monitoring setup, therefore, depends on the research question and resources available and is best identified in a team consisting of epidemiologists being familiar with the region and experts performing the wastewater study.

3.2 Analytical Approaches and Recommendations

Analysis of drug residues in wastewater poses an analytical challenge due to the complexity of the wastewater matrix and the low concentration levels of drugs in question. This holds especially true for NPS because consumption of the latter has not reached levels comparable to those of more frequently used classic illicit drugs. While the most common way to pre-treat wastewater is by means of filtration to remove larger particulates, centrifugation is also being used (Bade et al. 2017; González-Mariño et al. 2016b; Hernández et al. 2018 [open access]).

The rather complex matrix requires specific treatment steps. Target compounds can interact with the particulates in the sample, the sample bottle material, the materials used for the sample handling (Causanilles et al. 2017), and matrix components can interfere during the ionization phase before entering the mass spectrometer. These parameters can lead to suppression of the detector signal up to 80% (Bijlsma et al. 2013) when extracting 100 mL of sewage water influent. Several options are available to tackle these effects: matrix matched calibration, standard addition, or the addition of isotope-labeled internal standards prior to sample handling.

Sample preparation methods (aimed to remove interferences and to concentrate analytes) usually employ solid phase extraction (SPE) to extract drug residues from the liquid phase of wastewater and accelerated solvent extraction or microwave assisted extraction to extract the residues from the solid phase of wastewater (i.e., suspended particulate matter) (Baker and Kasprzyk-Hordern 2011; Evans et al. 2015; Křesinová et al. 2016; Petrie et al. 2016b). Sorbents most often used in SPE for illicit drugs are reversed phase (RP) polymers or mixed mode RP-cationic exchange materials. The extracts are then analyzed by means of ultrahigh performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). For chromatographic separation, a C18 column is typically used for classic illicit drugs, but other options have been shown to be successful for NPS analysis, such as polar endcapped C18 (Senta et al. 2015), biphenyl (Kinyua et al. 2016), pentafluorophenyl (Borova et al. 2015), and HILIC (Kinyua et al. 2015). The development of analytical equipment also offers the possibility of directly injecting large injection volumes (Bagnati and Davoli 2011; Berset et al. 2010; Chiaia et al. 2008).

Usually, triple quadrupole mass spectrometers are used for sensitive and selective quantitative multi-residue measurements of target analytes (Hernández et al. 2018 [open access]). As an example, Fig. 2 represents UHPLC-MS/MS chromatograms corresponding to the detection of mephedrone and butylone in influent wastewater. However, high resolution mass spectrometry (e.g., QTOF and Orbitrap) is gaining popularity due to its high selectivity and mass accuracy and its ability to not only quantify target drugs but also search for unknown compounds and facilitate retrospective search for analytes (Bijlsma et al. 2013; González-Mariño et al. 2016a; Hernández et al. 2011, 2018 [open access]). The search for the presence of NPS is often carried out through screening using a list of suspected and known analytes that is based on existing inventories, such as the EMCDDA Implementation Reports published annually within the European Union (EMCDDA 2016).

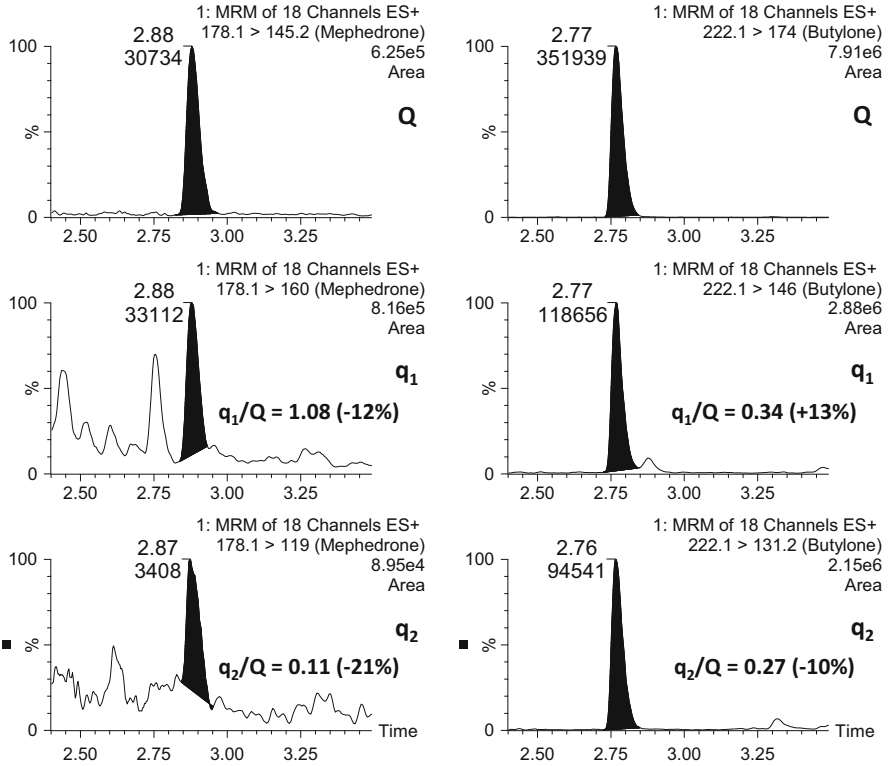

Fig. 2 UHPLC-MS/MS chromatograms corresponding to the positive finding of mephedrone (left) and butylone (right) in influent wastewater. **Q** quantification transition; $\mathbf{q_1}$ and $\mathbf{q_2}$ confirmation transitions. Retention times and ion ratios (q/Q) deviations were within tolerance limits (<0.1 min and <30%, respectively) in relation to reference standards, which allowed confirming the identity of the compounds in samples

Correct quantification of drug residues is of critical importance in wastewater analysis. Therefore, robust method validation, intra- and inter-day quality assurance, and comprehensive quantification protocols (van Nuijs et al. 2018) including labeled internal standards (preferably single isotope-labeled internal standards for each target compound) to correct for sample preparation and analysis derived errors, i.e., matrix effects, are now considered to be the norm. Several isotope-labeled NPS are commercially available (Borova et al. 2015).

The usage of chiral chromatography provides yet another dimension to the interpretation of wastewater-derived analysis since many NPS have one or more chiral centers. Chiral chromatography allows for verification of origins of biomarkers in wastewater (e.g., differentiation between consumption/direct exposure and other disposal routes, e.g., disposal of unused drugs) (Kasprzyk-Hordern and Baker 2012). This is due to stereoselective metabolism of chiral drugs taking place in humans. For example, MDMA is synthesized as a racemate. However,

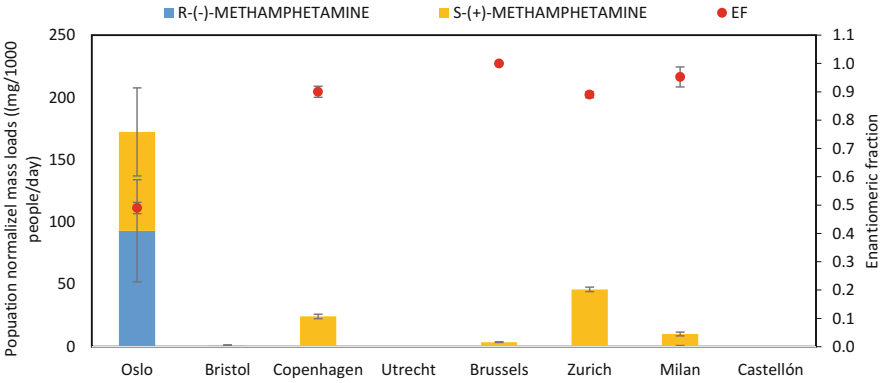

Fig. 3 Weekly average methamphetamine population-normalized mass loads and enantiomeric fraction values in a European monitoring campaign (modified from Castrignanò et al. 2018)

after its administration and stereoselective human metabolism, it is excreted with urine enriched with the (*R*)-(−)-enantiomer. Direct disposal of racemic MDMA as a result of police raid in the Netherlands (see Sect. 3.5) is one of the prime examples (Emke et al. 2014), where a chiral CBH column was used. Chiral chromatography can also help with verification of potency of drugs used in studied communities as seen in recent pan-European study investigating methamphetamine use across Europe. High loads of racemic methamphetamine were detected in Oslo. This is in contrast to other European cities where (*S*)-(+)-methamphetamine was the predominant enantiomer. This indicates different methods of methamphetamine synthesis and/or trafficking routes in Oslo, compared with the other cities tested (see Fig. 3) (Castrignanò et al. 2018).

3.3 Population Size Estimation

An accurate estimation of the size of the population that is served by a WWTP in order to normalize biomarker mass loads to the per capita level is currently one of the biggest bottlenecks of WBE. Accurate population size permits temporal and spatial comparisons to be made (Castiglioni et al. 2013; Daughton 2018). Until now, WBE studies used data that originated from public surveys (i.e., census data) and a wide array of demographic statistics to estimate the size of the population under investigation. However, this type of information is static and mostly captures only the de jure population (i.e., formal residents), whereas in WBE studies, it is more relevant to have knowledge on the de facto population, i.e., those being present in the WWTP catchment area, regardless of the location of their formal residence (Daughton 2012). Therefore, there is a need for alternative ways to estimating the de facto population of a WWTP catchment area.

Some research has been performed on the hydrochemical parameters that are routinely determined in the WWTPs (chemical oxygen demand (COD), biological oxygen demand (BOD), and total nitrogen and phosphorus) as markers of population size (Been et al. 2014; van Nuijs et al. 2011b). Been and co-workers showed promising results for normalization to NH_4^+–N load as dynamic measures used in WBE studies (Been et al. 2014). However, an important limitation of using hydrochemical parameters is that they often not only reflect human activities but that they can also be influenced by other external events, such as industrial or agricultural activities.

Further research into population estimation of a WWTP catchment area has been guided towards direct indicators of human activity and metabolism, i.e., naturally occurring and synthetic xenobiotics as well as products of endogenous metabolism in wastewater. However, to be a suitable candidate biomarker, several criteria have to be met (Daughton 2012; Gracia-Lor et al. 2017b):

1. Unique to human activity and excreted in substantial amounts
2. Low inter- and intra-individual variance in the daily excretion; excretion not affected by variables such as season, weather, and geographic location
3. Stable in wastewater during in-sewer transport and during storage until analysis

Several xenobiotics and endogenous compounds have been studied in this regard, such as pharmaceuticals, artificial sweeteners, nicotine, caffeine, creatinine, 5-hydroxyindoleacetic acid, cholesterol, cortisol, and androstenedione (Brewer et al. 2012; Chen et al. 2014; Lai et al. 2011, 2015; O'Brien et al. 2014, 2017; Senta et al. 2015). However, to date, none of the investigated population size markers have yet met all necessary criteria.

Recently, Thomas et al. proposed an alternative way based on mobile device-based activity to provide a dynamic way of the de facto population estimation which could be useful in WBE studies (Thomas et al. 2017). The authors concluded from their research that mobile device analytics might be promising since it addresses the uncertainties associated with both short- and long-term changes in population. In addition, it provides WBE scientists with population data that can be used to investigate and validate new (or improved) (hydro)chemical, hydrodynamic population markers of population, in cases where a mobile device monitoring option is not available.

3.4 Catchment and Sewer System Characterization

Besides the three most important variables to calculate population-normalized biomarker loads, that is: (1) biomarker concentration, (2) wastewater volume, and (3) population size, it is required to collect additional information about catchment characteristics. This is essential for data quality assurance and interpretation of data. This involves details on:

Technical	Nontechnical
• Type and operation of sewer systems • Sample collection (see Sect. 3.1) • Unusual operating conditions of sewer system	• Special events in the catchment (e.g., festivals and holidays)

Operators of WWTPs and sewer systems are the professionals who provide subsamples of their routinely collected samples, which they normally collect and analyze for standard parameters to confirm efficacy of the treatment process. These are the professionals who can also provide relevant details about the catchment and sewer system characteristics. Standardized questionnaires facilitate collection of these data. An example can be found in the supporting information of Castiglioni et al. (2013).

3.4.1 Type and Operation of Sewer Systems

In a separate sewer system, urban wastewater (from households and industry generated during irrespective of weather) and surface runoff (originating from precipitation) are collected in two different pipe systems. In contrast, in a combined sewer system, surface runoff during wet weather dilutes municipal wastewater. However, the increased wastewater flow results in the same illicit drug loads, assuming that illicit drugs are exclusively contained in the municipal wastewater as long as the WWTP can cope with the entire wastewater flow. If not, for example, during wet weather, a part of the wastewater can be discharged with no or only minor treatment into the environment and this part is not captured in the flow measurement in the influent of the WWTP. Potentially, during wet weather not all wastewater is arriving at the WWTP.

Pumps, detention tanks, and other special infrastructure can impact on the temporal pattern of pollutant loads and concentrations and influence the specification to obtain a representative sample (see Sect. 3.1 and cited literature therein). Abnormal operation in the sewer systems or the WWTP (e.g., maintenance) or normal operation during wet weather may imply that not all wastewater generated in the catchment arrives at the WWTP, or with a delay. Therefore, it is important to identify "normal" dry weather wastewater volumes and compare them with wastewater volumes measured during the monitoring period.

3.4.2 Special Events in the Catchment

Technical aspects related to sewer systems do not affect drug consumption, but can lead to undesirable consequences on sampling specification or observed biomarker loads (see above). If not specifically asked for from WWTP operators, this information is hidden and unknown to researchers applying WBE. In contrast, events such as holiday weeks, public holidays, festivals, sporting events, etc., can have two effects: (1) emergence of a different population size than normal (important for normalization of drug loads) and/or (2) different composition of the population. These nontechnical abnormalities are more obvious than the technical ones and therefore, not elaborated on in more detail. The quantification of the effect of the two aspects is difficult, but at least data can be flagged and compared to

baseline data for days where no such special events are known. Another special event that introduces further variables is represented by direct discharges of unconsumed drugs (see Sect. 3.5).

3.5 Direct Discharges of Unconsumed Drugs

One important issue that should not be overlooked in the evaluation of WBE data are the direct discharges into the sewer of biomarkers used for the community-wide drug use estimation. In the past 7 years, several types of evidence for direct discharges have become eminent.

The first signs of direct discharges of illicit drugs into sewer systems became apparent when monitoring the wastewater of Schiphol airport, a major international airport in the Netherlands (Bijlsma et al. 2012). The ratio of the levels of cocaine and its metabolite benzoylecgonine was not corresponding to commonly observed in-sewer ratios reflecting human metabolism, suggesting that drug traffickers were unloading their goods prematurely (Bijlsma et al. 2012). In another case reported in the UK, the direct disposal of an estimated 915 fluoxetine capsules was confirmed by chiral analysis (Petrie et al. 2016a).

The first multicity campaign in Europe executed in 2011 (Thomas et al. 2012) revealed another case of a direct discharge. The levels of MDMA observed in 2011 in the sewer of the city of Utrecht in the Netherlands were about 30 times higher than recorded by Bijlsma et al. (2012) in 2010 in the same city. Through the separation and quantification of the two MDMA enantiomers, it was concluded (Emke et al. 2014) that the MDMA observed in the wastewater had not passed the human body since the enantiomer ratio was racemic, whereas human metabolism changes the racemic composition to a higher contribution of the (*R*)-(−)-enantiomer (Moore et al. 1996). Information obtained from the police resulted in the conclusion that most likely under the pressure of a police raid, 30 kg of MDMA were dumped into the sewer close to the WWTP.

In the following years (2012–2017), during a European multicity monitoring exercise carried out by the Sewage Analysis CORe group Europe (SCORE), unexplained high levels of MDMA and amphetamine were encountered each year in the wastewater from the city of Eindhoven in the South of the Netherlands (Ort et al. 2014a; Thomas et al. 2012). The catchment area of the sewer system of Eindhoven is relatively large and includes many rural communities nearby. The southern part of the Netherlands is known for its production sites of MDMA and amphetamine (EMCDDA 2015). The illegal producers need to dispose of synthesis waste contaminated with final products as well as intermediate products and synthesis by-products. The synthesis waste is ranging from highly acidic ($pH < 1$) to highly basic ($pH > 10$) or it contains organic solvents which all pose a threat to relatively small WWTPs. A typical synthesis for the production of amphetamine results in 20–30 kg of chemical waste for 1 kg of amphetamine (EMCDDA 2015). Although the high levels of chemical waste, presumably from illegal syntheses of amphetamine or MDMA, disposed in the sewer system of

Eindhoven have not affected the functioning of its WWTP in the past years, it was impossible to calculate consumption figures for both illicit drugs.

In the Netherlands, a shift towards more professionalized laboratories has been reported. In 2014, a complete laboratory ordered in China was confiscated (Boerman et al. 2017). After mephedrone was banned in the EU at the end of 2010 following a risk assessment carried out under the auspices of the EMCDDA (2011), criminals could no longer import the drug directly and switched to local production of mephedrone for the international market. Also production sites for methamphetamine and captagon (fenethylline) were discovered in the Netherlands (Boerman et al. 2017). One drug that gained some popularity in specific subpopulations in the Netherlands was 4-fluoroamphetamine (4-FA) which is now a listed chemical in the Netherlands since 2017 (WHO 2017). Nowadays, 4-fluoromethamphetamine (4-FMA) appears to be gaining popularity. It is available through online shops and is marketed as an alternative for the now controlled 4-FA. Although the extent of illegal NPS production is not exactly known, it is expected that in the years to come direct discharges of the parent compounds and their synthesis waste products will be encountered.

In a case also reported in the South of the Netherlands described by Emke et al. (2018), it was shown that relatively small WWTPs are highly vulnerable to direct discharges of acidic waste into sewers that arise from drug synthesis. The active sludge of such WWTPs can become completely inactivated when exposed to the amounts of acidic waste dumped, resulting in malfunctioning or termination of the treatment process. Restoration of the process can only be done by renewed inoculation of active sludge. The study of Emke et al. (2018) also showed that synthesis markers (precursors, intermediates, and synthesis by-products such as benzylmethyl ketone, alpha-phenyl acetoacetamide, *N*-formylamphetamine, and 5-phenyl-4-methylpyrimidine), typically of the synthesis pathways employed, are usually detectable in the chemical waste discharged into the sewer system. The identification of specific synthesis markers in wastewater is not only useful to establish whether a direct discharge of waste has occurred but can also serve for forensics purposes to determine which (pre)precursors were used in the manufacturing of the drug.

3.6 Ethical Aspects and Communication (Media Attention)

WBE can be applied for different purposes. It was branded in the context of estimating illicit drug use, but it has also been the approach to quantify, e.g., the contribution of health care facilities to the load of pharmaceutically active ingredients in municipal wastewater (e.g., Langford and Thomas 2009; Ort et al. 2010a), alcohol or nicotine consumption (e.g., Baz-Lomba et al. 2016; Castiglioni et al. 2015; Gatidou et al. 2016; Reid et al. 2011; Senta et al. 2015), and other health parameters (e.g., obesity; Newton et al. 2015), in addition to NPS use

(e.g., Kinyua et al. 2015). Furthermore, numerous other potential applications can be thought of (Thomas and Reid 2011), such as sporting events (doping by athletes [Causanilles et al. 2018], but also changes in drug use in the general public, e.g., Gerrity et al. 2011).

As with many other research endeavors, it is highly recommended that researchers inform relevant partners about intentions of the study, particularly WWTP operators or owners of individual premises (e.g., hospitals, or prisons). If these stakeholders are not supportive, it can be difficult to obtain a wastewater sample. Before publication, they should also be proactively informed about the outcome of the study. From our experience, in most places, for most applications of WBE, no anonymization of data/location was required since no major ethical concerns arise when studies are carried out in the wastewater of large populations (Hall et al. 2012). It is impossible to identify individuals (respect for autonomy, i.e., consent and confidentiality) and there is no direct harm to participants (non-maleficence). In this setting, the approach also satisfies the principle of beneficence and distributive justice, since WBE can potentially inform on interventions aimed at improving public health and no social groups are singled out (Hall et al. 2012). However, this might be different, for example, if a socially disadvantaged suburb or district or individual premises (entertainment venues, prisons [e.g., Brewer et al. 2016; Postigo et al. 2011; Van Dyken et al. 2016], schools/colleges/universities [e.g., Burgard et al. 2013], and workplaces) are investigated, since indirect harms of WBE cannot be excluded. In such cases, potential policy responses (e.g., collective punishment in a prison) should be discussed with relevant stakeholders as part of the study, before carrying out the actual analyses. It should also be noted that the logistic and technical efforts to obtain representative samples from individual venues are substantially higher than collecting a sample from a WWTP (Ort 2014).

While factual reporting (method, accuracy, and potential benefit) by media to the general public is beneficial, negative media coverage can result in sensationalism and amplification of stigmatization (for more details, see Prichard et al. 2014). Typically, human research ethics committees have not required ethical review of WBE studies and, therefore, Prichard et al. (2014) recommended the development of ethical guidelines (see also SCORE 2017a). Special attention should be given to regions/groups where specific sensitivities may apply and how media communication of findings can be improved (e.g., asking journalists to send their article for comment before publication or avoiding quotations being taken out of context in a TV interview).

4 Applications of Wastewater-Based Epidemiology

Rapid progress in the field of WBE has been facilitated through successful international collaboration. The SCORE group, established in 2010, brought together experts working on illicit drugs analysis from several European countries (www.score-cost.eu) with the aim of undertaking international studies comparing illicit drug use between major European cities and evaluating different analytical

procedures being used in different labs. Supported by the EMCDDA, the group established a wider European network (now including >20 countries) that for the first time allowed an international study on the analysis of wastewater to be performed with the aim of estimating the use of illicit drugs on a European scale (Thomas et al. 2012).

Europe-wide monitoring campaigns are now undertaken for 1 week every year for amphetamine, cocaine, MDMA, and methamphetamine. This started with 19 cities in 11 countries in 2011 and covered over 80 WWTPs in 23 countries in 2017. Over the 7 years, more than 120 cities with a total population of approximately 61 million people were monitored at least once (including a few cities outside Europe investigated by associated partners) and 25 cities have participated in at least five of the campaigns (Fig. 4).

The most recent study in 2017 employed a 1-week monitoring period where samples from the wastewater of a total of over 36.7 million people were collected every day and analyzed by project partners. The results show distinct spatial differences across Europe with the data reported online by the EMCDDA (http://www.emcdda.europa.eu/activities/wastewater-analysis). For example, daily per capita loads of benzoylecgonine, the main excretion product of cocaine, ranged from "not quantifiable" (i.e., near zero) in, e.g., Cluj-Napoca (Romania) to over

Fig. 4 European monitoring campaigns: Up to 67 cities participated per year between 2011 and 2017 with a peak total population of approx. 38 million in 2015 (modified from SCORE 2017b)

400–800 mg/1,000 people/day in Amsterdam, Antwerp, London, and Zurich. Furthermore, differences within countries could be observed, with lower per capita loads in smaller towns compared to higher values in metropolitan areas. The analysis of methamphetamine revealed a completely different picture: Budweis and Prague, Piestany and Bratislava, Oslo, Turku, and Dresden, i.e., cities in Northern and Eastern Europe dominated the scene. Whereas spatial differences are obvious and findings from wastewater analyses were consistent with information held by epidemiologists in a quantitative manner, a comprehensive analysis of temporal changes will require a longer time series. Therefore, it is desirable that cities participate every year. To obtain more representative country-wide averages, it seems appropriate to expand the number of cities, also including small towns and villages (Ort et al. 2014a). In 2016, the European Commission contracted the WATCH consortium for a wastewater analysis report on the European stimulant illicit drug market.

In 2012, in order to develop transdisciplinary and cross-sectoral European research capability for the next generation of scientists working in the field of WBE, the group received funding from the European Commission to start a European Marie Curie Initial Training Network, SEWPROF ITN. SEWPROF ITN established interdisciplinary and cross-sectoral research capability and trained the next generation of scientists working in the new and exciting field of WBE. It developed and validated new, integrated tools towards public health monitoring at a community level based on innovative WBE techniques. SEWPROF ITN undertook the first Europe-wide profiling of community-wide health and lifestyle including oxidative stress (Ryu et al. 2016), exposure to pesticides (Rousis et al. 2017a), presence of synthetic cathinones and phenethylamines (Bade et al. 2017; González-Mariño et al. 2016b), and geographical differences in potency of methamphetamine use across Europe (Castrignanò et al. 2018). In order to facilitate international collaboration, the SCORE group received further EU funding in 2014 to establish a COST action (www.score-cost.eu). This was initiated in April 2014, developed and expanded the existing pan-European interdisciplinary network, bringing together experts from relevant disciplines interested in the application and development of the quantitative measurement of human biomarkers in sewage to evaluate lifestyle, health, and exposure at the community level.

Outside of Europe, Australia has led the way in establishing a National Wastewater Drug Monitoring Program (https://www.acic.gov.au/publications/intelligence-products/national-wastewater-drug-monitoring-program-report) that has measured the level of illicit drug consumption of around 60% of the Australian population every 3–4 months (dependent on location) since 2016. The Australian National Wastewater Drug Monitoring Program monitors 54 sites and 14 substances with the goal of providing concrete data on illicit drug consumption to inform policy in health, education, and law enforcement. The program has to date shown that methamphetamine is the most prevalent illicit drug tested in Australia, and that the opiates oxycodone and fentanyl exceed heroin consumption and the rise of 3,4-methylenedioxyamphetamine (MDA) as a tangible problem in some regional areas.

Outside of Europe and Australia, research projects have been performed in China, New Zealand, Costa Rica, Canada, and the USA although, to the best of the authors' knowledge, the establishment of formal monitoring programs has yet to be consolidated.

5 Wastewater-Based Epidemiology Beyond Traditional Illicit Drugs: New Psychoactive Substances and Public Health Monitoring

The NPS market is very dynamic as traditional drugs are quickly substituted with new alternatives. There have been in excess of 620 compounds reported to the EMCDDA early warning system up to 2016. A total of 423 individual substances were detected on the drug market in 2015 alone (EMCDDA-Europol 2016).

Data derived from the analysis of wastewater can provide quantitative information on NPS use, which can then be integrated with epidemiological information. Analysis of wastewater may in this way prove to be a useful tool in the development, management, and amendment of effective policies in the management of NPS and associated outcomes. The scale and dynamics of the NPS market do, however, present some significant challenges to wastewater scientists. The large number of individual substances and the relatively small size of the NPS market relative to traditional drugs make it difficult to identify and detect individual NPS in municipal wastewater (Reid and Thomas 2016). There is also a lack of (clinical) data from rigorous pharmacokinetic profiling which would provide necessary information on the identity and rates of excretion of the drugs and/or metabolites, which are critical for back-calculations from measured loads in sewers to the actual amounts of drugs used.

There are currently three key strategies for dealing with the challenge of NPS and wastewater. These include:

(a) Rigorous maintenance of target (NPS) databases to provide analytical chemists with the most up-to-date list of drugs that may be present in a given wastewater sample.
(b) Targeted analysis of wastewater from populations for whom the use of NPS is expected, such as from toilets at nightclubs and festivals. This is most easily achieved via analysis of pooled urine from pissoirs and portable toilets at and around the target venues.
(c) The use of nontargeted analytical methodologies, which allow for retrospective screening of new drugs after their inclusion in NPS databases.

These strategies have successfully been employed by NPS-Euronet which is an EU funded network (HOME/2014/JDRU/AG/DRUG/7086) tasked with developing an integrated chemical analytical epidemiological approach to improve the capacity to identify and assess new drugs (http://www.npseuronet.eu/). This network and other groups have detected numerous NPS in samples of pooled urine and wastewater (Bade et al. 2017; Borova et al. 2015; Chen et al. 2013;

González-Mariño et al. 2016b; Kinyua et al. 2016; Tscharke et al. 2016). The NPS-Euronet continues to work closely with authorities to provide information useful to the implementation and effective practice of prevention.

Wastewater analysis has proven invaluable in drug community-wide use estimation and is now evaluated in the context of identification of NPS. WBE has also a clear potential to revolutionize public health assessment at the community level (Reid et al. 2011; Ryu et al. 2015; Thomas and Reid 2011; Yang et al. 2015a, b). This is because wastewater reflects the health status of a population and surrounding environment as it pools endo- and exogenous biomarkers of that population. It provides opportunities to develop a wide range of innovative solutions to quickly and quantitatively assess patterns of factors related to health and disease within populations, while also providing a means of collecting complementary data for epidemiological and socioeconomic studies in order to undertake comprehensive evaluation of public health. As an example, Ryu et al. (2016) reported a Europe-wide monitoring of 8-iso-PGF2α (an oxidative stress biomarker). Increased levels of 8-iso-PGF2α were observed at the inner-city level correlating with the degree of urbanization and levels of nicotine use. Rousis et al. reported different levels of community-wide exposure to pyrethroid pesticides in eight Italian cities (Rousis et al. 2017b). Gracia-Lor et al. profiled caffeine use in ten European cities (Gracia-Lor et al. 2017a). Lopardo et al. identified new biomarkers of internal exposure to endocrine disruptors (Lopardo et al. 2017).

6 Conclusions

WBE has rapidly become a complementary tool, alongside existing epidemiology-based techniques, for providing objective, evidence-based estimates of illicit drug use. Adopted by EMCDDA, WBE now provides spatial and temporal drug use trends in Europe and is being rapidly implemented worldwide, taking Finland and Australia as prime examples. Ongoing developments in this field focus on: (1) comprehensive evaluation of uncertainties, (2) collation of long-term datasets on spatio-temporal drug usage trends in different geographical locations that will allow for comprehensive exposure assessment, and (3) new applications including an introduction of an early warning system for NPS. New applications of WBE, that are likely to transform public health monitoring, include an estimation of public exposure to food, environmental, and industrial chemicals as well as tracking the development and spread of infectious disease in vulnerable communities.

References

Bade R, Bijlsma L, Sancho JV, Baz-Lomba JA, Castiglioni S, Castrignanò E, Causanilles A, Gracia-Lor E, Kasprzyk-Hordern B, Kinyua J, McCall AK, van Nuijs ALN, Ort C, Plósz BG, Ramin P, Rousis NI, Ryu Y, Thomas KV, de Voogt P, Zuccato E, Hernández F (2017) Liquid chromatography-tandem mass spectrometry determination of synthetic cathinones and phenethylamines in influent wastewater of eight European cities. Chemosphere 168:1032–1041

Bagnati R, Davoli E (2011) Analytical methods for the detection of illicit drugs in wastewaters and surface waters. In: Castiglioni S, Zuccato E, Fanelli R (eds) Chapter 3 in illicit drugs in the environment. Wiley, New York, NY

Baker DR, Kasprzyk-Hordern B (2011) Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry. J Chromatogr A 1218(44):7901–7913

Baz-Lomba JA, Salvatore S, Gracia-Lor E, Bade R, Castiglioni S, Castrignanò E, Causanilles A, Hernandez F, Kasprzyk-Hordern B, Kinyua J, McCall AK, Van Nuijs A, Ort C, Plósz BG, Ramin P, Reid M, Rousis NI, Ryu Y, de Voogt P, Bramness J, Thomas K (2016) Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. BMC Public Health 16(1):1–11

Been F, Rossi L, Ort C, Rudaz S, Delémont O, Esseiva P (2014) Population normalization with ammonium in wastewater-based epidemiology: application to illicit drug monitoring. Environ Sci Technol 48(14):8162–8169

Been F, Bijlsma L, Benaglia L, Berset J-D, Botero-Coy AM, Castiglioni S, Kraus L, Zobel F, Schaub MP, Bücheli A, Hernández F, Delémont O, Esseiva P, Ort C (2016a) Assessing geographical differences in illicit drug consumption – a comparison of results from epidemiological and wastewater data in Germany and Switzerland. Drug Alcohol Depend 161:189–199

Been F, Esseiva P, Delémont O (2016b) Analysis of illicit drugs in wastewater – is there an added value for law enforcement? Forensic Sci Int 266:215–221

Been F, Schneider C, Zobel F, Delémont O, Esseiva P (2016c) Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland. Int J Drug Policy 36:33–42

Berset JD, Brenneisen R, Mathieu C (2010) Analysis of llicit and illicit drugs in waste, surface and lake water samples using large volume direct injection high performance liquid chromatography – electrospray tandem mass spectrometry (HPLC-MS/MS). Chemosphere 81(7):859–866

Bijlsma L, Emke E, Hernández F, de Voogt P (2012) Investigation of drugs of abuse and relevant metabolites in Dutch sewage water by liquid chromatography coupled to high resolution mass spectrometry. Chemosphere 89(11):1399–1406

Bijlsma L, Emke E, Hernández F, de Voogt P (2013) Performance of the linear ion trap Orbitrap mass analyzer for qualitative and quantitative analysis of drugs of abuse and relevant metabolites in sewage water. Anal Chim Acta 768(1):102–110

Boerman F, Grapendaal M, Nieuwenhuis F, Stoffers E (2017) Nationaal dreigingsbeeld 2017 – Georganiseerde criminaliteit (in Dutch). National Police, Zoetermeer

Borova VL, Gago-Ferrero P, Pistos C, Thomaidis NS (2015) Multi-residue determination of 10 selected new psychoactive substances in wastewater samples by liquid chromatography-tandem mass spectrometry. Talanta 144:592–603

Brewer AJ, Ort C, Banta-Green CJ, Berset JD, Field JA (2012) Normalized diurnal and between-day trends in illicit and legal drug loads that account for changes in population. Environ Sci Technol 46(15):8305–8314

Brewer AJ, Banta-Green CJ, Ort C, Robel AE, Field J (2016) Wastewater testing compared with random urinalyses for the surveillance of illicit drug use in prisons. Drug Alcohol Rev 35(2):133–137

Burgard DA, Fuller R, Becker B, Ferrell R, Dinglasan-Panlilio MJ (2013) Potential trends in attention deficit hyperactivity disorder (ADHD) drug use on a college campus: wastewater analysis of amphetamine and ritalinic acid. Sci Total Environ 450–451:242–249

Castiglioni S, Zuccato E, Crisci E, Chiabrando C, Fanelli R, Bagnati R (2006) Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry. Anal Chem 78(24):8421–8429

Castiglioni S, Bijlsma L, Covaci A, Emke E, Hernández F, Reid M, Ort C, Thomas KV, van Nuijs ALN, de Voogt P, Zuccato E (2013) Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. Environ Sci Technol 47(3):1452–1460

Castiglioni S, Senta I, Borsotti A, Davoli E, Zuccato E (2015) A novel approach for monitoring tobacco use in local communities by wastewater analysis. Tob Control 24(1):38–42

Castrignanò E, Yang Z, Bade R, Baz-Lomba JA, Castiglioni S, Causanilles A, Covaci A, Gracia-Lor E, Hernandez F, Kinyua J, McCall AK, van Nuijs ALN, Ort C, Plósz BG, Ramin P, Rousis NI, Ryu Y, Thomas KV, de Voogt P, Zuccato E, Kasprzyk-Hordern B (2018) Enantiomeric profiling of chiral illicit drugs in a pan-European study. Water Res 130:151–160

Causanilles A, Baz-Lomba JA, Burgard DA, Emke E, González-Mariño I, Krizman-Matasic I, Li A, Löve ASC, McCall AK, Montes R, van Nuijs ALN, Ort C, Quintana JB, Senta I, Terzic S, Hernandez F, de Voogt P, Bijlsma L (2017) Improving wastewater-based epidemiology to estimate cannabis use: focus on the initial aspects of the analytical procedure. Anal Chim Acta 988:27–33

Causanilles A, Nordmann V, Vughs D, Emke E, de Hon O, Hernández F, de Voogt P (2018) Wastewater-based tracing of doping use by the general population and amateur athletes. Anal Bioanal Chem 410(6):1793–1803

Chen C, Kostakis C, Irvine RJ, White JM (2013) Increases in use of novel synthetic stimulant are not directly linked to decreased use of 3,4-methylenedioxy-N-methylamphetamine (MDMA). Forensic Sci Int 231(1):278–283

Chen C, Kostakis C, Gerber JP, Tscharke BJ, Irvine RJ, White JM (2014) Towards finding a population biomarker for wastewater epidemiology studies. Sci Total Environ 487:621–628

Chiaia AC, Banta-Green C, Field J (2008) Eliminating solid phase extraction with large-volume injection LC/MS/MS: analysis of illicit and legal drugs and human urine indicators in US wastewaters. Environ Sci Technol 42(23):8841–8848

D'Ascenzo G, Di Corcia A, Gentili A, Mancini R, Mastropasqua R, Nazzari M, Samperi R (2003) Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Sci Total Environ 302(1):199–209

Daughton CG (2012) Real-time estimation of small-area populations with human biomarkers in sewage. Sci Total Environ 414:6–21

Daughton CG (2018) Monitoring wastewater for assessing community health: sewage chemical-information mining (SCIM). Sci Total Environ 619–620:748–764

EMCDDA (2011) Report on the risk assessment of mephedrone in the framework of the Council Decision on new psychoactive substances. Publications Office of the European Union, Luxembourg

EMCDDA (2015) Synthetic drug production in Europe, European Monitoring Centre for Drugs and Drug Addiction. Publications Office of the European Union, Luxembourg

EMCDDA (2016) Assessing illicit drugs in wastewater: advances in wastewater-based drug epidemiology. Office for Official Publications of the European Communities, Lisbon, Luxembourg

EMCDDA-Europol (2016) EMCDDA – Europol 2016 annual report on the implementation of Council Decision 2005/387/JHA. http://www.emcdda.europa.eu/system/files/publications/4724/TDAN17001ENN_PDFWEB.pdf archived with webciation.org under http://www.webcitation.org/6xH50d2Pr

Emke E, Evans S, Kasprzyk-Hordern B, de Voogt P (2014) Enantiomer profiling of high loads of amphetamine and MDMA in communal sewage: a Dutch perspective. Sci Total Environ 487(1):666–672

Emke E, Vughs D, Kolkman A, de Voogt P (2018) Wastewater-based epidemiology generated forensic information: amphetamine synthesis waste and its impact on a small sewage treatment plant. Forensic Sci Int 286:e1–e7. https://doi.org/10.1016/j.forsciint.2018.03.019

Evans SE, Davies P, Lubben A, Kasprzyk-Hordern B (2015) Determination of chiral pharmaceuticals and illicit drugs in wastewater and sludge using microwave assisted extraction, solid-phase extraction and chiral liquid chromatography coupled with tandem mass spectrometry. Anal Chim Acta 882:112–126

Gatidou G, Kinyua J, van Nuijs ALN, Gracia-Lor E, Castiglioni S, Covaci A, Stasinakis AS (2016) Drugs of abuse and alcohol consumption among different groups of population on the Greek Island of Lesvos through sewage-based epidemiology. Sci Total Environ 563–564:633–640

Gerrity D, Trenholm RA, Snyder SA (2011) Temporal variability of pharmaceuticals and illicit drugs in wastewater and the effects of a major sporting event. Water Res 45(17):5399–5411

González-Mariño I, Gracia-Lor E, Bagnati R, Martins CPB, Zuccato E, Castiglioni S (2016a) Screening new psychoactive substances in urban wastewater using high resolution mass spectrometry. Anal Bioanal Chem 408(16):4297–4309

González-Mariño I, Gracia-Lor E, Rousis NI, Castrignanò E, Thomas KV, Quintana JB, Kasprzyk-Hordern B, Zuccato E, Castiglioni S (2016b) Wastewater-based epidemiology to monitor synthetic cathinones use in different European countries. Environ Sci Technol 50(18):10089–10096

Gracia-Lor E, Zuccato E, Castiglioni S (2016) Refining correction factors for back-calculation of illicit drug use. Sci Total Environ 573(suppl C):1648–1659

Gracia-Lor E, Rousis NI, Zuccato E, Bade R, Baz-Lomba JA, Castrignanò E, Causanilles A, Hernández F, Kasprzyk-Hordern B, Kinyua J, McCall A-K, van Nuijs ALN, Plósz BG, Ramin P, Ryu Y, Santos MM, Thomas K, de Voogt P, Yang Z, Castiglioni S (2017a) Estimation of caffeine intake from analysis of caffeine metabolites in wastewater. Sci Total Environ 609:1582–1588

Gracia-Lor E, Castiglioni S, Bade R, Been F, Castrignanò E, Covaci A, González-Mariño I, Hapeshi E, Kasprzyk-Hordern B, Kinyua J, Lai FY, Letzel T, Lopardo L, Meyer MR, O'Brien J, Ramin P, Rousis NI, Rydevik A, Ryu Y, Santos MM, Senta I, Thomaidis NS, Veloutsou S, Yang Z, Zuccato E, Bijlsma L (2017b) Measuring biomarkers in wastewater as a new source of epidemiological information: current state and future perspectives. Environ Int 99:131–150

Hall W, Prichard J, Kirkbride P, Bruno R, Thai PK, Gartner C, Lai FY, Ort C, Mueller JF (2012) An analysis of ethical issues in using wastewater analysis to monitor illicit drug use. Addiction 107(10):1767–1773

Harman C, Reid M, Thomas KV (2011) In situ calibration of a passive sampling device for selected illicit drugs and their metabolites in wastewater, and subsequent year-long assessment of community drug usage. Environ Sci Technol 45(13):5676–5682

Hernández F, Bijlsma L, Sancho JV, Díaz R, Ibáñez M (2011) Rapid wide-scope screening of drugs of abuse, prescription drugs with potential for abuse and their metabolites in influent and effluent urban wastewater by ultrahigh pressure liquid chromatography-quadrupole-time-of-flight-mass spectrometry. Anal Chim Acta 684(1–2):96–106

Hernández F, Castiglioni S, Covaci A, de Voogt P, Emke E, Kasprzyk-Hordern B, Ort C, Reid M, Sancho JV, Thomas KV, van Nuijs AL, Zuccato E, Bijlsma L (2018) Mass spectrometric strategies for the investigation of biomarkers of illicit drug use in wastewater. Mass Spectrom Rev 37:258–280. https://doi.org/10.1002/mas.21525 (open access)

Kasprzyk-Hordern B, Baker DR (2012) Estimation of community-wide drugs use via stereoselective profiling of sewage. Sci Total Environ 423:142–150

Kinyua J, Covaci A, Maho W, McCall AK, Neels H, van Nuijs ALN (2015) Sewage-based epidemiology in monitoring the use of new psychoactive substances: validation and application of an analytical method using LC-MS/MS. Drug Test Anal 7(9):812–818

Kinyua J, Negreira N, Miserez B, Causanilles A, Emke E, Gremeaux L, de Voogt P, Ramsey J, Covaci A, van Nuijs ALN (2016) Qualitative screening of new psychoactive substances in pooled urine samples from Belgium and United Kingdom. Sci Total Environ 573:1527–1535

Křesinová Z, Linhartová L, Petrů K, Krejčová L, Šrédlová K, Lhotský O, Kameník Z, Cajthaml T (2016) Method for analysis of psychopharmaceuticals in real industrial wastewater and groundwater with suspended organic particulate matter using solid phase extraction disks extraction and ultra-high performance liquid chromatography/time-of-flight mass spectrometry. J Chromatogr A 1440:15–22

Lai FY, Ort C, Gartner C, Carter S, Prichard J, Kirkbride P, Bruno R, Hall W, Eaglesham G, Mueller JF (2011) Refining the estimation of illicit drug consumptions from wastewater analysis: co-analysis of prescription pharmaceuticals and uncertainty assessment. Water Res 45(15):4437–4448

Lai FY, Bruno R, Leung HW, Thai PK, Ort C, Carter S, Thompson K, Lam PKS, Mueller JF (2013) Estimating daily and diurnal variations of illicit drug use in Hong Kong: a pilot study of using wastewater analysis in an Asian metropolitan city. Forensic Sci Int 233(1–3):126–132

Lai FY, Anuj S, Bruno R, Carter S, Gartner C, Hall W, Kirkbride KP, Mueller JF, O'Brien JW, Prichard J, Thai PK, Ort C (2015) Systematic and day-to-day effects of chemical-derived population estimates on wastewater-based drug epidemiology. Environ Sci Technol 49(2):999–1008

Langford KH, Thomas KV (2009) Determination of pharmaceutical compounds in hospital effluents and their contribution to wastewater treatment works. Environ Int 35(5):766–770

Lopardo L, Cummins A, Rydevik A, Kasprzyk-Hordern B (2017) New analytical framework for verification of biomarkers of exposure to chemicals combining human biomonitoring and water fingerprinting. Anal Chem 89(13):7232–7239

McCall A-K, Bade R, Kinyua J, Lai FY, Thai PK, Covaci A, Bijlsma L, van Nuijs ALN, Ort C (2016) Critical review on the stability of illicit drugs in sewers and wastewater samples. Water Res 88:933–947

McCall AK, Palmitessa R, Blumensaat F, Morgenroth E, Ort C (2017) Modeling in-sewer transformations at catchment scale – implications on drug consumption estimates in wastewater-based epidemiology. Water Res 122:655–668

Moore KA, Mozayani A, Fierro MF, Poklis A (1996) Distribution of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) stereoisomers in a fatal poisoning. Forensic Sci Int 83(2):111–119

Newton RJ, McLellan SL, Dila DK, Vineis JH, Morrison HG, Murat Eren A, Sogin ML (2015) Sewage reflects the microbiomes of human populations. MBio 6(2):e02574–e02514

O'Brien JW, Thai PK, Eaglesham G, Ort C, Scheidegger A, Carter S, Lai FY, Mueller JF (2014) A model to estimate the population contributing to the wastewater using samples collected on census day. Environ Sci Technol 48(1):517–525

O'Brien JW, Banks APW, Novic AJ, Mueller JF, Jiang G, Ort C, Eaglesham G, Yuan Z, Thai PK (2017) Impact of in-sewer degradation of pharmaceutical and personal care products (PPCPs) population markers on a population model. Environ Sci Technol 51(7):3816–3823

Ort C (2014) Quality assurance/quality control in wastewater sampling. In: Zhang C, Mueller JF, Mortimer M (eds) Quality assurance & quality control of environmental field samples. Future Science, London

Ort C, Gujer W (2006) Sampling for representative micropollutant loads in sewer systems. Water Sci Technol 54(6–7):169–176

Ort C, Lawrence MG, Reungoat J, Eaglesham G, Carter S, Keller J (2010a) Determining the fraction of pharmaceutical residues in wastewater originating from a hospital. Water Res 44(2):605–615

Ort C, Lawrence MG, Reungoat J, Mueller JF (2010b) Sampling for PPCPs in wastewater systems: comparison of different sampling modes and optimization strategies. Environ Sci Technol 44(16):6289–6296

Ort C, Lawrence MG, Rieckermann J, Joss A (2010c) Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review. Environ Sci Technol 44(16):6024–6035

Ort C, van Nuijs ALN, Berset JD, Bijlsma L, Castiglioni S, Covaci A, de Voogt P, Emke E, Fatta-Kassinos D, Griffiths P, Hernández F, González-Mariño I, Grabic R, Kasprzyk-Hordern B, Mastroianni N, Meierjohann A, Nefau T, Östman M, Pico Y, Racamonde I, Reid M, Slobodnik J, Terzic S, Thomaidis N, Thomas KV (2014a) Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. Addiction 109(8):1338–1352

Ort C, Eppler JM, Scheidegger A, Rieckermann J, Kinzig M, Sörgel F (2014b) Challenges of surveying wastewater drug loads of small populations and generalizable aspects on optimizing monitoring design. Addiction 109(3):472–481

Petrie B, Youdan J, Barden R, Kasprzyk-Hordern B (2016a) New framework to diagnose the direct disposal of prescribed drugs in wastewater – a case study of the antidepressant fluoxetine. Environ Sci Technol 50(7):3781–3789

Petrie B, Youdan J, Barden R, Kasprzyk-Hordern B (2016b) Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-performance liquid chromatography tandem mass spectrometry. J Chromatogr A 1431:64–78

Postigo C, de Alda ML, Barceló D (2011) Evaluation of drugs of abuse use and trends in a prison through wastewater analysis. Environ Int 37(1):49–55

Prichard J, Hall W, de Voogt P, Zuccato E (2014) Sewage epidemiology and illicit drug research: the development of ethical research guidelines. Sci Total Environ 472:550–555

Ramin P, Brock AL, Causanilles A, Valverde-Pérez B, Emke E, de Voogt P, Polesel F, Plósz BG (2017) Transformation and sorption of illicit drug biomarkers in sewer biofilms. Environ Sci Technol 51(18):10572–10584

Reid M, Thomas KV (2016) New psychoactive substances: analysis and site-specific testing. In: Assessing illicit drugs in wastewater: advances in wastewater-based drug epidemiology, EMCDDA insights, vol 22. Publications Office of the European Union, Luxembourg, pp 57–65

Reid MJ, Langford KH, Mørland J, Thomas KV (2011) Analysis and interpretation of specific ethanol metabolites, ethyl sulfate, and ethyl glucuronide in sewage effluent for the quantitative measurement of regional alcohol consumption. Alcohol Clin Exp Res 35(9):1593–1599

Rieckermann J, Neumann M, Ort C, Huisman JL, Gujer W (2005) Dispersion coefficients of sewers from tracer experiments. Water Sci Technol 52(5):123–133

Rousis NI, Gracia-Lor E, Zuccato E, Bade R, Baz-Lomba JA, Castrignanò E, Causanilles A, Covaci A, de Voogt P, Hernàndez F, Kasprzyk-Hordern B, Kinyua J, McCall AK, Plósz BG, Ramin P, Ryu Y, Thomas KV, van Nuijs A, Yang Z, Castiglioni S (2017a) Wastewater-based epidemiology to assess pan-European pesticide exposure. Water Res 121:270–279

Rousis NI, Zuccato E, Castiglioni S (2017b) Wastewater-based epidemiology to assess human exposure to pyrethroid pesticides. Environ Int 99:213–220

Ryu Y, Reid MJ, Thomas KV (2015) Liquid chromatography–high resolution mass spectrometry with immunoaffinity clean-up for the determination of the oxidative stress biomarker 8-iso-prostaglandin F2alpha in wastewater. J Chromatogr A 1409:146–151

Ryu Y, Gracia-Lor E, Bade R, Baz-Lomba JA, Bramness JG, Castiglioni S, Castrignanò E, Causanilles A, Covaci A, de Voogt P, Hernandez F, Kasprzyk-Hordern B, Kinyua J, McCall AK, Ort C, Plósz BG, Ramin P, Rousis NI, Reid MJ, Thomas KV (2016) Increased levels of the oxidative stress biomarker 8-iso-prostaglandin F 2α in wastewater associated with tobacco use. Sci Rep 6:39055

SCORE (2017a) Ethical guidelines. PDF archived with WebCitation.org, available here: http://www.webcitation.org/6wkvm8g1l (original webpage: http://score-cost.eu/wp-content/uploads/sites/118/2016/11/WBE-ethical-guidelines-FINAL-March-2016-.pdf)

SCORE (2017b) Sewage analysis CORE group Europe http://score-cost.eu/

Senta I, Gracia-Lor E, Borsotti A, Zuccato E, Castiglioni S (2015) Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment. Water Res 74:23–33

Thomaidis NS, Gago-Ferrero P, Ort C, Maragou NC, Alygizakis NA, Borova VL, Dasenaki ME (2016) Reflection of socioeconomic changes in wastewater: licit and illicit drug use patterns. Environ Sci Technol 50(18):10065–10072

Thomas KV, Reid MJ (2011) What else can the analysis of sewage for urinary biomarkers reveal about communities? Environ Sci Technol 45(18):7611–7612

Thomas KV, Bijlsma L, Castiglioni S, Covaci A, Emke E, Grabic R, Hernández F, Karolak S, Kasprzyk-Hordern B, Lindberg RH, Lopez de Alda M, Meierjohann A, Ort C, Pico Y, Quintana JB, Reid M, Rieckermann J, Terzic S, van Nuijs ALN, de Voogt P (2012) Comparing illicit drug use in 19 European cities through sewage analysis. Sci Total Environ 432(0):432–439

Thomas KV, Amador A, Baz-Lomba JA, Reid M (2017) Use of mobile device data to better estimate dynamic population size for wastewater-based epidemiology. Environ Sci Technol 51(19):11363–11370

Tscharke BJ, Chen C, Gerber JP, White JM (2016) Temporal trends in drug use in Adelaide, South Australia by wastewater analysis. Sci Total Environ 565:384–391

Van Dyken E, Lai FY, Thai PK, Ort C, Bruno R, Hall W, Kirkbride KP, Mueller JF, Prichard J (2016) Challenges and opportunities in using wastewater analysis to measure drug use in a small prison facility. Drug Alcohol Rev 35(2):138–147

van Nuijs ALN, Castiglioni S, Tarcomnicu I, Postigo C, de Alda ML, Neels H, Zuccato E, Barcelo D, Covaci A (2011a) Illicit drug consumption estimations derived from wastewater analysis: a critical review. Sci Total Environ 409(19):3564–3577

van Nuijs ALN, Mougel J-F, Tarcomnicu I, Bervoets L, Blust R, Jorens PG, Neels H, Covaci A (2011b) Sewage epidemiology – a real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium. Environ Int 37(3):612–621

van Nuijs ALN, Lai FY, Been F, Andres-Costa MJ, Barron L, Baz-Lomba JA, Berset J-D, Benaglia L, Bijlsma L, Burgard D, Castiglioni S, Christophoridis C, Covaci A, de Voogt P, Emke E, Fatta-Kassinos D, Hernandez F, Gerber C, González-Mariño I, Grabic R, Gunnar T, Kannan K, Karolak S, Kasprzyk-Hordern B, Kokot Z, Krizman-Matasic I, Li A, Li X, Löve ASC, Lopez de Alda M, Meyer MR, Oberacher H, O'Brien J, Quintana JB, Reid M, Schneider S, Sadler Simoes S, Thomaidis NS, Thomas K, Yargeau V, Ort C (2018) Multi-year interlaboratory exercises for the analysis of illicit drugs and metabolites in wastewater: development of a quality control system. Trends Anal Chem 103:34–43. https://doi.org/10.1016/j.trac.2018.03.009

WHO (2017) 4-Fluoroamphetamine (4-FA) critical review report, agenda item 4.3. http://www.who.int/medicines/access/controlled-substances/CriticalReview_4FA.pdf. Accessed 8 Feb 2018 and archived on http://www.webcitation.org/6x4zm6eJV

Yang Z, d'Auriac MA, Goggins S, Kasprzyk-Hordern B, Thomas KV, Frost CG, Estrela P (2015a) A novel DNA biosensor using a ferrocenyl intercalator applied to the potential detection of human population biomarkers in wastewater. Environ Sci Technol 49(9):5609–5617

Yang Z, Kasprzyk-Hordern B, Frost CG, Estrela P, Thomas KV (2015b) Community sewage sensors for monitoring public health. Environ Sci Technol 49(10):5845–5846

Zuccato E, Chiabrando C, Castiglioni S, Bagnati R, Fanelli R (2008) Estimating community drug abuse by wastewater analysis. Environ Health Perspect 116(8):1027–1032

Zuccato E, Castiglioni S, Tettamanti M, Olandese R, Bagnati R, Melis M, Fanelli R (2011) Changes in illicit drug consumption patterns in 2009 detected by wastewater analysis. Drug Alcohol Depend 118(2–3):464–469

Zuccato E, Castiglioni S, Senta I, Borsotti A, Genetti B, Andreotti A, Pieretti G, Serpelloni G (2016) Population surveys compared with wastewater analysis for monitoring illicit drug consumption in Italy in 2010–2014. Drug Alcohol Depend 161:178–188