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ABSTRACTS

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Monitoring New Psychoactive Substance Markets

A taste for novel psychoactive substances: wastewater analysis from 12 countries over 2019–20 and 2020–21

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Introduction: Novel Psychoactive Substances (NPS) are a complex addition to the international drug market. Their initial designation as 'legal highs' intimated safe and licit consumption in place of conventional illicit drugs such as ecstasy, cocaine and cannabis. However, (co-)consumption of these compounds has possibly led to hospitalization and fatalities. Due to the proliferation of these compounds – by January 2021, more than 1000 NPS had been reported to the United Nations Office of Drugs and Crime Early Warning Advisory from 126 countries covering all continents – it is imperative to monitor these compounds. In this work, influent wastewater samples were collected to provide an international snapshot of NPS use over the New Year period in 2019/20 and 2020/21, times synonymous with parties and increased drug use. A second campaign allowed the possibility of seeing how NPS trends have changed and potentially predicting where and when such NPS will emerge next. ***Methods***: Influent wastewater was collected from 14 sites in eight countries (Australia, United States, New Zealand, Italy, Spain, Norway, China, and the Netherlands) from 26 December 2019 – 3 January 2020, and from 24 sites in 10 countries (Australia, New Zealand, China, Spain, Italy, Canada, United States, Fiji, Republic of Korea and Belgium) from 25 December 2020 – 3 January 2021. These sites included small towns and large cities as well as places with a known influx of holiday makers. Samples were either sent directly to Australia for analysis or first loaded onto solid-phase extraction cartridges. All samples were analysed with two liquid chromatography - mass spectrometry methods. One employed a quantitative targeted method for 21 NPS and the other a qualitative high-resolution mass spectrometry screening method with a database of more than 200 NPS. ***Results***: A total of 15 NPS were found in the samples from 2019/20: mephedrone, 3-methylmethcathinone, N-ethylpentylone, methcathinone, ethyl one, MDPV, pentylone and methylone were able to be quantified, while 4-chloromethcathinone, 4-fluoromethamphetamine, mitragynine, acetyl fentanyl and eutylone were qualitatively found. In the 2020/21 samples, 11 NPS were quantified: 3-methylmethcathinone, ethylone, eutylone, mephedrone, methcathinone, methiopropamine, methoxetamine, methylone, N-ethylpentylone, pentylone and PMA. 7-hydroxymitragynine was also qualitatively seen. Some geographical trends were observed, with mephedrone confined to Australia and New Zealand, 3-methylmethcathinone in Europe and eutylone in Australia, New Zealand and North America. In addition, some were only seen in specific countries such as 4-fluoroamphetamine and 4-fluoromethamphetamine in the Netherlands and (7-hydroxy) mitragynine in the United States. ***Conclusions***: This work has shown the utility of wastewater analysis to monitor community loads of NPS. A snapshot into the consumption of NPS in 12 countries was made by analysing samples over the 2019-20 and 2020-21 New Year periods. By continuing to monitor these substances temporally and spatially, it may be possible to predict the onset of emerging NPS.

A survey of drugs seized at New Zealand music festivals: The Eutylone Boogaloo

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Introduction: Surveys of the drugs seized at music festivals in New Zealand (NZ) were conducted over the 2018-2019 and 2020-2021 summer festival periods. These surveys were conducted to provide insight into drug trends at music festivals and how these have changed over time. Here, the results from both festival periods will be presented, compared, and discussed. ***Methods***: Samples were submitted from NZ Police seizures and drug

amnesty bins at music festivals across the 2018-19 and 2020-2021 summer festival period. Samples were screened for the presence of controlled substances using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared spectroscopy (FT-IR). As a comparison to common on-site drug screening methods, a handheld Raman spectrometer was additionally used to compare to routine laboratory testing methods. Approximate dosages were estimated from the weight of substances in capsules, paper wraps and cannabis cigarettes. ***Results***: MDMA and eutylone were the most prevalent drugs, followed by cannabis plant. The 2020-2021 summer festival period saw a dramatic increase in eutylone detections compared to the 2018-2019 survey period. Other substances detected were methamphetamine, ketamine, LSD and cocaine. No synthetic cannabinoids, GBL or fentanyl-type substances were detected in any of the samples. Doses of MDMA and other substances present in capsules ranged from 70 to 100 milligrams. The handheld screening device was successful at identifying only a limited number of samples, highlighting the limitations of such testing methods. ***Conclusion***: Analysis of drug seizures from music festivals indicated a changing drug trend with a dramatic increase in eutylone in the most recent survey, highlighting the need for a continual monitoring program. The comparison between handheld screening devices and laboratory testing suggested a collaborative approach was needed between on-site testing agencies and more in-depth laboratory analysis.

Emerging non-fentanyl-related synthetic opioids in the United States

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Introduction: Over 950 new psychoactive substances (NPS) were identified in the past decade, which are being monitored domestically in the United States. NPS among the synthetic opioids class pose particular threats to the public health and safety in the United States and countries around the world. ***Methods***: We will present recent data on synthetic opioids in the United States, focusing on an apparent transition away from fentanyl-related compounds (FRC) to other synthetic opioids. The data primarily comes from the Drug Enforcement Administration's (DEA) National Forensic Laboratory Information System (NFLIS). NFLIS-Drug provides accurate, chemically and/or otherwise verified data which is used to identify emerging drugs as well as diversion, trafficking, and abuse patterns geographically and over time in support of federal, state, and international drug policy initiatives. We will also consider data from multiple other sources, including, but not limited to, the United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory on NPS (EWA), the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) European Union Early Warning System on NPS (EWS), and Health Canada Drug Analysis Service (DAS), as they are key to early identification of emergent drug patterns in the United States. Finally, we will highlight the timelines of some select substances that have emerged in the United States illicit drug market. ***Results***: The impact on the rate of new NPS emerging in the United States illicit drug market is evaluated following multiple individual control actions and a temporary control action on fentanyl-related substances (FRS) as a class. The number of FRS reports to NFLIS-Drug have noticeably decreased in recent years, whereas reports of new non-FRC synthetic opioids have increased in recent years. Select substances highlighted will include new FRS and benzimidazoles. ***Conclusions***: Early identification of emerging threats in the illicit market requires close collaboration, efficient communication, and the new modality of early warning systems to mine, analyze and model data.

Novel synthetic opioid trends from NPS Discovery 2018–2021

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Introduction: With the emergence of fentanyl in US markets prior to 2010 and subsequent efforts by regulatory authorities to control it, the United States experienced an influx of fentanyl analogs originating from chemists

manufacturing both legacy fentanyl analogs from the Janssen 1960s patents as well as their own variations on that theme. Fentanyl analogs appeared in the early 2010s and had passed their peak by the end of 2018, largely due to national and international control efforts. ***Methods***: Data and reports available from NPS Discovery (Center for Forensic Science Research and Education, United States) were queried and consolidated to evaluate trends for novel synthetic opioids between 2018 and 2021. The results were compared with available patent and research literature. ***Results/Discussion***: As early as 2016, there was increasing evidence of clandestine chemists producing new drugs with known mu opioid receptor agonist effects, including drugs in the cyclohexyl-N-methylbenzamide subclass beginning with U-47700. Related analogs which appeared later in the cycle were methylenedioxy-U-47700, U-48800, and U-49900 in 2018, N-methyl U-47931E in 2019, and 3,4-difluoro-U-47700, and N-ethyl U-47700, in 2020. These U-series drugs were either described in patents from the Upjohn Company from the 1970's or were newly manufactured analogs. U-47700 was the predominant member of this class with potency only around 10% that of fentanyl. U-47700 appeared in many cases along with fentanyl or other analogs, especially furanylfentanyl, during its peak prevalence. Other U-series analogs appeared in much smaller numbers and did not appear to enter the mainstream recreational drug supply. In 2019, 2-methyl AP-237 was detected in a shipment intercepted at an international express mail facility entering the US. This drug was first described in pharmaceutical research in the 1970's in Japan and was the first member in its series of new opioids to be detected in the US. Buccinazine (AP-237) was also detected in 2019, and *para*-methyl AP-237 and AP-238 were detected in 2020. 2-Methyl AP-237 has been the most encountered drug of this subclass. The next major subclass of synthetic opioids contributing to deaths were the 2-benzyl benzimidazoles, beginning with isotonitazene in 2019, followed by metonitazene in 2020 and several others in 2021. Most of these drugs originated from pharmaceutical research in the 1950's in Switzerland, and were known to research chemical users through the first decade of the 2000's. This subclass has shown the greatest recent proliferation, with additional analogs including butonitazene, etodesnitazene, flunitazene, N-pyrrolidino etonitazene, protonitazene and metodesnitazene. Unlike previously mentioned subclasses, several members of the 2-benzyl benzimidazoles attain relative potency greater than fentanyl (including N-pyrrolidino etonitazene > isotonitazene > protonitazene > metonitazene > fentanyl). In total, members of this subclass have contributed to over 400 deaths. In 2020, between of the prevalence of isotonitazene and metonitazene, there was a brief period of popularity for brorphine, a piperidinyl benzimidazolone. Brorphine appeared in pharmaceutical research published in 2018. It quickly became a frequent finding in opioid related deaths between June and December 2020, with few detections remaining in September 2021. In total, brorphine was reported in over 220 deaths.

Synthetic psychoactive substances advertised on one cryptomarket over a one-year period

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Introduction: The past decade has seen an unprecedented number of Novel Psychoactive Substances (NPS) emerge on the global drug market. Over the same period, Darknet markets, or cryptomarkets, have become increasingly important for the distribution of illicit psychoactive substances. However, the exact number and volume of the synthetic substances listed on these markets remains understudied. This paper describes the type of synthetic psychoactive substances available on one cryptomarket over a one-year period. ***Methods***: Listings from the White House Market (WHM) were collected weekly between 25th August 2020 and 23rd August 2021. The eDarkTrends2 Named Entity Recognition algorithm was deployed to identify synthetic substances from seven categories of substances: Benzodiazepine, Cathinone, Dissociative, Phenylamine (Amphetamine-type), Synthetic Cannabinoid Receptor Agonist (SCRA), Novel Synthetic Opioid, and Tryptamine. ***Results***: 1,452,013 listings were collected from WHM through 52 crawling sessions. 35 benzodiazepines were identified among 13.8% of the total listings (n=199,983/1,452,013); 22 cathinones were identified in 16.3% of the total listings (n=236,709/1,452,013); 16 dissociatives were detected among 6.6% of the total listings (n=95,283/1,452,013); 28 phenylamines were identified in 14.7% of the total listings (n=212,748/1,452,013); 30 SCRA were identified in 0.6% of the total listings

(n=8,472/1,452,013); 17 novel synthetic opioids were detected among 0.7% of the total listings (n=10,219/1,452,013); and 16 synthetic tryptamines were identified in 4.8% of the total listings (n=69,431/1,452,013). ***Conclusions***: Collected data indicate a large volume and variety of synthetic substances advertised on one cryptomarket. The ability to identify consistently synthetic psychoactive substances offers the possibility to timely monitor the emergence of NPS and underscores the value of cryptomarkets for early warning systems.

Parallel Session I **Wednesday November 17, 2021**

Early Warning Systems and Toxicovigilance

Drug seizure monitoring in prisons as early-warning systems of new psychoactive substance use: synthetic cannabinoids and other emerging psychoactive substances

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Introduction: New psychoactive substance (NPS) use in prisons is common in many jurisdictions. Synthetic cannabinoid receptor agonists (SCRAs) infused papers are of particular concern. Early warning systems and effective monitoring programs track the emergence and fluctuations in prevalence of new substances, informing evidence-based legislative change and supply and harm reduction measures. ***Methods***: We have analyzed >1300 non-attributable drug seizures in Scottish prisons using complimentary analytical techniques (IMS, GC-MS, LC-HRAMS, NMR). Data is presented for >560 SCRA-infused papers seized between June 2018 and November 2021. ***Results***: Potent tert-leucinamide (ADB)-type SCRAs increased in prevalence in 2021. ADB-BUTINACA was detected in January 2021, within weeks becoming the most prevalent SCRA in Scottish prisons; ADB-4en-PINACA was detected December 2020 to February 2021; ADB-HEXINACA was detected April to May 2021. We consider the reason for this market change and likely implications of the recent SCRA analogue ban introduced by China. We report the emergence/re-emergence of several SCRAs seeking to circumvent that legislation. ***Conclusions***: We have demonstrated the utility of responsive long-term prison drug seizure monitoring programs and the need to accurately predict drug market changes; to reduce future supply and harms; and focus research on substances of the greatest concern.

NPS Discovery — Evolution of an open-access drug early-warning system

A.J. Krotulski, B.K. Logan, CFSRE, United States of America

Introduction: NPS Discovery launched in 2018 as an avenue for rapid and timely dissemination of vital information regarding the detection of novel psychoactive substances (NPS) in the United States (US), filling the void for a program that did not exist nationally. The genesis of the program involved the development the new drug monographs which included chemical information, a brief description, and analytical data. These documents continue to serve as notification that new NPS are present in the US recreational drug supply, allowing scientists and practitioners to respond accordingly in their respective jurisdictions. Since 2018, NPS Discovery has grown exponentially to become a premier open-access drug early warning system by utilizing an evidence-based approach to lead the development of additional high impact reports for real-time action. Also in 2018, NPS Discovery began an initiative to track emerging drug trends through the re-analysis of authentic forensic casework samples, including both biological samples and raw drug materials. This effort has continued through 2021 and has allowed for the production of quarterly trend reports for each NPS subclass and public alerts to rapidly notify stakeholders of drug threats based on increasing positivity and prevalence. In 2020, NPS Discovery unveiled two new initiatives with public health and clinical partners, showcasing newly acquired drug checking data and expanded toxicology testing on patients in emergency department settings, respectively. Most recently in 2021, NPS Discovery introduced a nation-wide, multi-jurisdictional effort to develop recommendations for scope of testing involving NPS. Additionally, our program has undertaken various research studies relating to NPS, including monitoring of drug use fora and gray market vendor sites and assessments of pharmacology and toxicity. ***Methods***: Analysis was conducted using a SCIEX TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer (LC-QTOF-MS), a Waters Vevo TQ-S micro tandem mass spectrometer (LC-MS/MS), and an Agilent 5975 gas chromatography mass spectrometer (GC-MS). ***Results***: Since 2018, NPS Discovery has produced 106 new drug monographs to alert the emergence of 31 opioids, 24 cannabinoids, 24 stimulants, 13 hallucinogens, 6 benzodiazepines, 6 opioid

precursors, and 2 miscellaneous drugs. Trend analysis in forensic samples has shown the emergence and proliferation of new generations of opioids linked to scheduling of fentanyl-related substances. Beginning in 2020, an exponential increase in benzodiazepine positivity was observed, with high rates of incidence with fentanyl and other opioids. Turnover of cannabinoids had continued, leading up to the recent detection of new generation cannabinoids post Chinese class-wide scheduling. Changes among stimulant and hallucinogen subclasses were observed to be slower and less volatile. ***Conclusion***: Pairing of data from public health and public safety realms has allowed NPS Discovery to triangulate knowledge and information in manners not previously available in US. While our program was initially designed with forensic toxicology workflows in mind, it has suited well for analysis of samples collected among other field of forensic science and other fields outside of the forensic space. Additionally, the NPS Discovery model and its associated reports are now consumed internationally for comparison of global drug markets and over NPS impacts.

Type of NPS and the consumer profile in the Canary Islands

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Introduction: NPS are psychoactive substances that mimic the effects of traditional illegal drugs, but whose outlawing is much slower than their introduction and distribution in markets. In recent years their consumption has increased in Western countries, especially among young individuals, due to the accessibility that the internet allows for its purchase and the existing legal vacuum. NPS detection in patients treated in hospital emergencies is currently null due to limitations in analytical techniques. The objective of this study was to know the type of NPS and the consumer profile of these substances in the Canary Islands, through their detection in intoxicated and treated patients in the emergency hospital services of the Canary Island Health Service. ***Methods***: Observational descriptive study of detection and identification of NPS in urine samples from 106 patients (older than 14 years) treated by intoxication or drug use in the emergency hospital services of 3 reference hospitals of the Canary Islands from 1 October 2019 to 31 January 2021. The samples were analyzed using a novel detection system (biochip Array Technology, Randox) in the Toxicology Laboratory of the ULPGC. ***Results***: NPS were detected in 62 cases (58.5% of the samples) with an average age of 34.1 years (SD 12.09). 61.3% of the positive cases were male. Except for the tryptamine group (more detected in males, p=0.02), no differences were found according to sex or age, among the positive cases. Ultrapotent opioids and new benzodiazepines were detected in more than 20% of the samples and piperacins and cathinones in about 15% of them. Synthetic cannabinoids were detected in 5% of the cases studied. 34.9% of the cases analyzed were positive for both NPS and classical drugs. Significantly, synthetic cathinones were most frequently found in patients in the capital area of Tenerife (Hospital de la Candelaria, p = 0.008), while tryptamines were mainly found in the Dr. Negrín Hospital. Synthetic cannabinoids and phenylethylamines were only detected in the capital area of Tenerife (HUC and Hospital de la Candelaria). ***Conclusions***: This study allows us to know for the first time in Spain the NPS present in a sample of patients intoxicated by drug use in the Hospital Emergency Services of the Canary Islands. 58% of the samples presented NPS, more than was reflected in the national and European consumption surveys. The most frequently detected NPS are ultra-potent opiates (fentanyl, carfentanil, acetylfentanyl and U47700), present in almost 27% of the samples, which may mean that the opioid abuse epidemic in the United States may be beginning to occur in our country. Prospective studies are necessary, without selection bias and with a greater number of hospitals and subjects, to know the consumption profile and the clinical repercussions of the use of NPS in the Canary Islands.

Pharmaco-toxicological Effects of (±)cis-4,4'-DMAR and (±)trans-4,4'-DMAR: Neuro-behavioural, Physiological, Immunohistochemical and Metabolic Studies in Mice

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Introduction: 4,4'-Dimethylaminorex (4,4'-DMAR) is a synthetic stimulant structurally similar to 4-methylaminorex (4-MAR) and aminorex sold in the illicit drug market. It has been found in Europe since the end of 2012 as in tablets or powder, and it has been involved in several intoxications and deaths. Up to now there is little information regarding the pharmaco-toxicological effects of 4-4'-DMAR and in particular those of its (±)*cis* and (±)*trans* stereoisomers and their co-administration in racemic mixture. ***Methods***: Therefore, the aim of this study is to investigate the effects of the systemic administration of the single (±)*cis* (0.1-60 mg/kg) and (±)*trans* (30 and 60 mg/kg) stereoisomers and of their co-administration ((±)*cis* at 1, 10 or 60 mg/kg + (±)*trans* at 30 mg/kg) in mice on physiological and behavioural parameters. Moreover, to highlight possible neurotoxic mechanisms we investigate the effect of 4,4'-DMAR on the expression of markers of oxidative/nitrosative stress (8-OHdG, iNOS, NT and NOX2), apoptosis (Smac/DIABLO and NF-κB) and heat shock proteins (HSP27, HSP70, HSP90) in cerebral cortex. ***Results***: Our study demonstrated that (±)*cis* stereoisomer dose-dependently induces psychomotor agitation, sweating, salivation, hyperthermia, stimulated aggression, convulsions and death. Conversely, the (±)*trans* stereoisomer was ineffective. Their co-administration results in a worsening of the toxic effects caused by (±)*cis* stereoisomer. This trend of responses was confirmed by histopathological analysis on the cortex, suggesting a potential neurotoxic effect of (±)*cis* and (±)*cis*+(±)*trans* administration. Finally, we investigated the potential mechanism underlying the potentiation of the toxic effects of the (±)*cis* stereoisomer when co-administered with the (±)*trans* stereoisomer by studying the urinary excretion. ***Conclusion***: The excretion study showed that the (±)*trans* stereoisomer reduces the metabolism of the (±)*cis* form and increases its amount in the urine, that possibly reflects its increased plasma levels and therefore the worsening of its toxicity.

Parallel Session II **Wednesday November 17, 2021**

Policy and Legislation

Scheduling and its impact on the emergence of NPS

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Introduction: Novel psychoactive substances (NPS) have continued to proliferate and remain a global phenomenon more than a decade after they first NPS appeared on the illicit drug market. The fast-paced NPS market is driven by a number of factors, some of which are associated with legal recourse such as national and international scheduling actions of both the drug itself and its precursors. This presentation will focus on impacts associated with scheduling actions, including a review to case positivity following scheduling and appearance of new substances following the scheduling of other drugs within the same class. ***Methods***: National and international scheduling actions were reviewed in conjunction with drug detection rates in both seized drug and toxicology casework. ***Results***: In 2018, China announced the scheduling actions to control two precursor chemicals used in the production of illicit fentanyl, including 4-anilinopiperidine (4-ANPP). Following the scheduling action, fentanyl positive cases containing 4-ANPP were relatively low. However, beginning in mid-May 2019 there was a significant uptick in the number of 4-ANPP positive results. Simultaneously, the detection of acetylfentanyl began to drop and had fallen by 50% by the end of the year. Both of these shifts occurred while the number of fentanyl positive results remained relatively stable. Related to synthetic cannabinoids, 5F-MDMB-PICA was first identified in our casework in October 2018. The intent to schedule came in December 2018, and the drug was temporarily scheduled in April 2019. 5F-MDMB-PICA positivity steadily increased, despite scheduling actions, and peak positivity wasn't observed until January 2020. However, with the announcement of temporary scheduling of 5F-MDMB-PICA, MDMB-4en-PINACA emerged in the US with the first detection occurring in July 2019. Similar patterns have been observed with novel synthetic opioids. Isotonitazene was first detected in case samples in November 2019. The US intent to schedule was issued in June 2020 and one month following (July 2020), brorphine was first identified in toxicology cases. N-ethylpentylone was first detected in August 2017 and scheduled in August 2018, which resulted in a steady decline in case positivity. Comparatively, eutylone, a drug which is not currently directly scheduled in the US has persisted and remains the NPS stimulant that is most frequently encountered drug in our casework. ***Conclusions***: Scheduling actions are designed to eliminate illicit substances from the market and have proven to be effective in reducing the supply of NPS that have already emerged. However, new substances are still quickly introduced to the market as their successors, therefore, increased monitoring of drug intelligence sources is recommended when notices of intent to schedule are made to provide insight as to what may be coming next in this dynamic market.

Legal impasse on the use of Ketum (Mitragyna speciosa) in Malaysia

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Introduction: The leaves from the medicinal plant which is known as "Ketum" or "Kratom" (Mitragyna speciosa) have been traditionally used for its purported curative properties, and as a panacea for illicit substance use among people who use drugs (PWUDs) in Malaysia. It has widespread use despite impacts for those caught for possessing and distributing ketum leaves, which can include prosecution in Malaysia. This paper discusses the persisting legal impasse regarding ketum use in Malaysia. ***Methods***: We used legal documents, published articles and newspaper reports on ketum to construct this paper. ***Results***: As a perennial evergreen tree, ketum can be seen growing wildly in rural communities in Malaysia. Ketum and its psychoactive alkaloid, mitragynine, is currently regulated under the Poisons Act of 1952. Though, the law does not prohibit cultivation, it criminalizes ketum possession, sales, import and export. Owing to its pervasive use and its alleged potential as a 'gateway drug', law-enforcement agencies have been pressuring the government to introduce harsh penalties to control ketum cultivation by

including it in the Dangerous Drugs Act of 1952, alongside drugs like heroin, cocaine opiates and amphetamine-type stimulant (ATS). The debate to criminalise ketum was brought to the attention of parliamentarians twice in the last six years but drew mixed reactions from lawmakers. The end result was a call for further studies and dialogues to find an amicable solution. Meanwhile, law enforcement agencies are pressing ketum cultivators to grow other crops, while settlers occupying government land have been threatened with a revocation of land use rights if they continue to plant ketum. In the midst of the raging policy debate surrounding the cultivation of ketum, PWUDs claim to have benefited significantly from ketum consumption, while studies documenting these claims have proliferated not only in Malaysia but also in neighboring regions like Thailand. Matters have taken an interesting turn with Thailand decriminalizing ketum in late August 2021 and Malaysian authorities reacting by tightening surveillance along their shared borders. ***Conclusions***: Given the impasse in the ketum policy debate, scientific studies to evaluate its potential to address the illicit substance use problem remains in limbo and underfunded, while people who use ketum (PWUK) continue to be burdened by law enforcers seeking to suppress its possession and use.

Controlled new psychoactive substances in New Zealand

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Introduction: The emergence of new psychoactive substances into the global drug market has presented challenges for effective drug legislation and enforcement. One approach to prohibition uses controlled drug analogue legislation, which involves assessing the structural similarity of new substances compared to listed controlled drugs, as opposed to the new substance being specifically listed in legislation itself. An issue arises of there being no clear definition for what constitutes similarity between two substances, and as such, there is a level of subjectivity in any decision made. This paper outlines the current method for considering new psychoactive substances as potential controlled drug analogues, as well as a proposed novel approach to this assessment process. ***Methods***: The authors propose an alternative approach for assessing similarity between two substances, which involves an objective and reproducible similarity scoring mechanism. ***Results***: The similarity between two substances can be expressed as a value, which can then be used to create thresholds for determining when two substances might be considered similar or substantially similar in a legal context. Subsequently, a new substance can be trialled in theoretical or actual receptor binding studies to determine the extent to which that new substance may cause physiological effects. In this way the chemical similarity can be correlated with pharmacological activity. ***Conclusions***: Ultimately, it is the potential for harm that a new substance poses that is important from a harm reduction perspective. This research looks at how the chemical similarity of a new substance might be used to estimate its psychoactivity and be used as an indicator for the potential harm that a new substance might cause.

Parallel Session III **Thursday November 18, 2021**

NPS Pharmacology and Toxicology

Synthesis, functional evaluation and molecular docking of NBOME and NBF positional isomers

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Introduction: Serotonergic psychedelics have continuously comprised a substantial portion of the over 1000 New Psychoactive Substances (NPS) reported so far. Despite their large structural variety, they share the activation of the serotonin 2A receptor (5-HT2AR) as a main pharmacological mechanism. Within the group of serotonergic psychedelics, three structural subgroups can be recognized: ergolines (such as LSD, lysergic acid diethylamide), tryptamines (with psilocybin as the prototypical substance), and phenylalkylamines (e.g. mescaline). The derivatives and structure-activity relationships of the latter group have been extensively explored in PiHKAL. However, for the more recent N-benzyl derivatives of those phenylalkylamines, such as the –NBOMe, -NBOH, and –NBF substances, this thorough characterization is still lagging behind. ***Methods***: Therefore, isomers of 25H-NBOMe and 25H-NBF with 2 methoxy groups on different positions of the phenyl ring of the phenethylamine core structure were synthesized and functionally characterized. The former group of substances was additionally docked into a model of the 5-HT2AR. Functional characterization was carried out via the in vitro NanoBiT® functional complementation assay, monitoring the recruitment of either β-arrestin 2 (βarr2) or miniGαq to the 5-HT2AR, hence enabling the calculation of potency and efficacy values for each of the isomers. ***Results***: When looking at the name of the positional isomers, the numbers denote the positions of the phenyl ring of the phenethylamine moiety at which a methoxy group was introduced: e.g. 24H-NBOMe carries methoxy groups at positions 2 and 4. For both the –NBOMe and the –NBF isomers, the 24H- isomers were the most potent, followed by the 25H- and 26H isomers, with 23H-NBOMe/NBF having a considerably lower potency. The least potent isomers were those with 34H- and 35H- substitutions. When looking at the in vitro efficacies, the substitution patterns of the 24H-, 25H-, and 26H- isomers resulted consistently in the highest values. For the –NBOMe group, additionally, 34H-NBOMe was one of the more efficacious substances, with 35H-NBOMe and 23H-NBOMe having lower efficacies. On the other hand, for the –NBF substances, 23H-NBF was more efficacious than 35H and 34H-NBF. The molecular modeling hypothesized different interactions of the methoxy groups of the –NBOMe isomers with amino acid residues in the ligand binding pocket of the 5-HT2AR. ***Conclusions***: Together, these data propose an important role for the methoxy group at position 2 of the phenyl ring of the phenethylamine moiety of –NBOMes and –NBFs for their in vitro activity. This finding is consistent with conclusions for the 2C-X counterparts without N-benzyl substituent.

Activity profiling of recent non-fentanyl synthetic opioids via three different μ-opioid receptor activation assays

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Introduction: Between 2015 and 2019, the total share of new synthetic opioids (NSOs) as a proportion of all new psychoactive substances (NPS) has quadrupled, rendering NSOs one of the fastest growing groups of NPS. Amid this highly dynamic opioid landscape, insight into the pharmacology of NSOs has become an important tool to estimate the harm potential of newly emerging drugs. In this work, we determined the μ-opioid receptor (MOR) affinity and activation potential of a panel of recent non-fentanyl NSOs. The use of three different MOR activation assays allows detailed profiling of the in vitro mechanism of action (and, hence, potential toxicity) of NSOs. ***Methods***: Seven NSOs (N-ethyl-U-47700, 3,4-difluoro-U-47700, U-47931E/bromadoline, 2,4-difluoro-U-48800, U-62066/spiradoline, 2F-viminol, ketobemidone) were newly evaluated at MOR. Their MOR affinity was determined via [3H]-DAMGO binding in rat brain tissue homogenates. MOR activation potential was studied at different levels

of receptor signaling using three functional assays. The first two assays are based on the functional complementation of a split nanoluciferase enzyme (NanoBiT®, Promega). In short, activation of MOR, fused to one part of the nanoluciferase, leads to recruitment of either β-arrestin 2 or mini-Gαi, fused to the complementing part. This restores the enzymatic activity, producing a bioluminescent signal upon addition of a substrate. The third assay measures the downstream increase in levels of intracellular Ca2+ following MOR activation (AequoScreen®). ***Results***: Several of the studied opioids have recently emerged on the recreational drug market or have been the subject of discussions among drug users on online platforms. The most active compounds were ketobemidone (EC50 32.8-528 nM; Emax 105-271%, relative to hydromorphone) and N-ethyl-U-47700 (EC50 = 241-767 nM; Emax = 139-247%). The same opioids showed the strongest MOR affinity. In general, most of the other NSOs only weakly activated MOR in the three assays, with EC50 values in the high nM to µM range. 2F-viminol (EC50 = 2.2-4.5 µM; Emax = 21.2-61.5%) and U-47931E/bromadoline (EC50 = 0.55-2.9 µM; Emax = 52.8-85.9%) were partial agonists compared to hydromorphone, and maximum receptor activation was not reached for 2,4-difluoro-U-48800 (EC50 > 22 µM). Our data further highlight the importance of considering the specific assay characteristics upon interpretation of potency and efficacy values. ***Conclusions***: The increasing presence of synthetic opioids on the recreational drug market continues to pose a substantial threat to public health. While in vitro research at MOR offers a first realistic estimation of the potential danger these compounds may evoke, one should be aware of the influence of distinct assay characteristics on the obtained results.

Structure-activity relationship of cumyl derived indole and indazole SCRAs: evaluation of alkylic and cyclic tails

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Introduction: Over the years, synthetic cannabinoid receptor agonists (SCRAs) have become the largest group of new psychoactive substances (NPS) monitored by the European Monitoring Centre for Drug and Drug Addiction. The complex molecular structures of many synthetic cannabinoids can be categorized into four pharmacophoric elements required for receptor binding (core, hydrophobic tail, linker, head group), with a critical role being played by the tail. Within a panel of related SCRAs, the determination of structure-activity relationship (SAR) is important to understand the effects of structural alterations for the prediction of the pharmacological properties of novel SCRAs. ***Methods***: SCRAs carrying a cumyl head group have been reported since 2014. Cumyl-PICA, cumyl-BUTICA, and cumyl-PINACA were initially found in seized powder samples. Cumyl-CBMICA and its indazole counterpart, encompassing cyclic tails were identified next. Cumyl-CBMICA was the first SCRA to feature a cyclobutylmethyl subunit as a pendant moiety from the heterocyclic core. Therefore, we evaluated here a panel of cumyl-derived indole and indazole SCRAs, encompassing alkyl or cyclic hydrophobic tails, to determine the SAR for cumyl-derived SCRAs. Included in the tested panel are previously reported or published cumyl-related compounds with butyl-, pentyl-, cyclobutylmethyl- and cyclohexylmethyl tails, accompanied by newly synthesized cumyl-derived indoles and indazoles encompassing cyclopropylmethyl- and cyclopentylmethyl tails, which are the first SCRAs encompassing such moieties as pharmacophoric subunit. ***Results***: The panel of 12 cumyl-derived indole- and indazole-3-carboxamides was evaluated using an in-house CB1 reporter assay monitoring β-arrestin 2 recruitment. Concentration-dependent curves were obtained for all tested compounds. EC50 and Emax values were derived as measures of potency and efficacy (relative to Emax of JWH-018), respectively. All studied cumyl derivatives acted as agonists at the CB1 receptor, however, substantial differences in potency (EC50 = 2.55-337nM) and efficacy (Emax = 63.8-206%) could be observed. Previously reported SAR regarding higher CB1 activity for indazole SCRAs compared to their indole counterparts was confirmed. In addition, in line with findings from several research groups, the pentyl tail was associated with the highest CB1 activity. The activity of the SCRAs encompassing cyclic tails decreased according to a decreasing number of carbons forming the cyclic moiety, with only cumyl-CPrMICA showing less activity than the reference compound JWH-018. ***Conclusion***: In conclusion, both an increasing alkyl

tail length (up to pentyl) and an increasing bulkiness of a cyclic tail result in increased CB1 activation in a panel of cumyl SCRAs.

Preclinical abuse liability assessment of tianeptine using intracranial self-stimulation in rats

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Introduction: Tianeptine is an atypical antidepressant medication that acts as an agonist at the mu-opioid receptor (MOR). In the United States, it is neither approved by the Food and Drug Administration as a therapeutic agent nor federally controlled by the Drug Enforcement Administration. In recent years, unregulated tianeptine has been sold in U.S. stores, and it has been abused at supratherapeutic doses. The abuse of this "gas station heroin" has resulted in hospitalizations and death. We conducted an abuse liability assessment of tianeptine using an intracranial self-stimulation (ICSS) procedure in rats. ***Methods***: ICSS was conducted in adult male Sprague-Dawley rats with electrodes targeting the medial forebrain bundle. Drug-naïve rats were trained to depress a lever to receive electrical brain stimulation at a fixed amplitude and variable frequency in test components consisting of ten one-minute frequency trials. Following training, the effects of intraperitoneal injection of tianeptine on ICSS were determined. In this procedure, brain stimulation produces a frequency-dependent increase in ICSS response rates, drugs of abuse further increase (or facilitate) ICSS responding, and MOR agonists like morphine produce stronger abuse-related ICSS facilitation after repeated daily treatment than after acute treatment. To evaluate the degree to which tianeptine might produce morphine-like abuse potential, studies were conducted in three phases. (1) To evaluate effectiveness of acute tianeptine to produce abuse-related ICSS facilitation, tianeptine (1.0 - 32 mg/kg) or saline vehicle was evaluated using ten-minute test components collected 10, 20, 30, 40, and 110 minutes post-administration (N=6). (2) To evaluate MOR mediation of acute tianeptine effects, a separate cohort of rats (n=3) was tested at a dose of 32 mg/kg tianeptine after pre-treatment with saline or the MOR antagonist naltrexone (0.1 mg/kg, s.c.). (3) To evaluate the degree to which repeated tianeptine might enhance abused-related ICSS facilitation produced either by itself or by morphine, the effects of saline and sequential increasing doses of tianeptine (3.2 – 32 mg/kg) on ICSS were determined before and after 7-day daily exposure to either 10 or 32 mg/kg tianeptine (n=7 per group). On day 9, rats underwent a similar sequential-dosing procedure using increasing doses of morphine (0.32 – 3.2 mg/kg). ***Results***: Acute 32 mg/kg tianeptine produced robust but transient ICSS depression that peaked after 10 min and dissipated after 40 min. Acute 10 mg/kg tianeptine produced initial ICSS depression followed by significant but delayed ICSS facilitation at 30 min. Effects of 1.0 and 3.2 mg/kg tianeptine were negligible. Naltrexone blocked ICSS depression by 32 mg/kg tianeptine. Repeated treatment with neither 10 nor 32 mg/kg/day tianeptine significantly enhanced abuse-related ICSS depression either by tianeptine itself or by morphine, although the higher 32 mg/kg/day tianeptine dose did produce modest tolerance to tianeptine-induced ICSS depression. ***Conclusions***: Tianeptine produced weak evidence for abuse potential, producing ICSS facilitation after only one dose (10 mg/kg) at one time point (30 min after administration). Moreover, repeated tianeptine failed to increase expression of abuse-related ICSS facilitation by either tianeptine itself or by morphine. The more prominent effect was ICSS depression, which was naltrexone reversible as with other MOR agonists. These results suggest that tianeptine is low-potency short-acting MOR agonist with limited abuse potential but significant potential for behavioral depression.

Systematic *in vitro* and estimated *in vivo* pharmacokinetics studies on synthetic cannabinoid receptor agonists as an aid to interpretation of toxicological casework data

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Introduction: The synthetic cannabinoid receptor agonist (SCRA) market constantly evolves; individual compounds emerge and their prevalence fluctuates in response to legislative changes, making interpretation of analytical toxicological data challenging. We present a systematic *in vitro* approach to increase understanding of the pharmacokinetics of SCRAs. **Methods**: Physico-chemical measurements and *in vitro* pharmacokinetic studies using pooled human liver microsomes (pHLM) and pooled cryopreserved human hepatocytes (pHHeps) were conducted on 22 SCRAs, including emerging leucinamide indazole-3-carboxamide SCRAs (e.g. ADB-BUTINACA) and other SCRA structural classes (e.g. 5F-MDA-19, 5F-3,5-PFUPPYCA). ***Results***: Experimentally derived lipophilicity data (Log D7.4) ranged from 2.8 to 5.0 and all SCRAs tested were highly protein bound. Most tested SCRAs were cleared rapidly in vitro particularly the valine methyl ester SCRAs (e.g. AMB-FUBINACA, 5F-AMB-PINACA, 5F-EMB-PICA), although some (e.g. 5F-3,5-PFUPPYCA) were cleared considerably more slow. The presence of certain structural features determined both the rate of in vitro clearance and the site of biotransformation. ***Conclusions***: SCRAs are often rapidly metabolised *in vitro* but are highly protein bound *in vivo* and therefore predicted *in vivo* hepatic clearance is considerably slower than calculated in vitro intrinsic clearance. This is likely to give rise to longer detection windows in blood and urine than might otherwise be expected.

Parallel Session IV **Thursday November 18, 2021**

NPS Use Settings, Experience and Trends (I)

A systematic review of (pre)clinical studies on the therapeutic potential and safety profile of kratom in humans

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Introduction: Kratom (Mitragyna speciosa) is a tropical plant traditionally used as an ethnomedicinal remedy for several conditions in South East Asia. Despite the increased interest in its therapeutical benefits in Western countries, little scientific evidence is available to support such claims, and existing data remains limited to kratom's chronic consumption. Our study aims to investigate (pre)clinical evidence on the efficacy of kratom as a therapeutic aid and its safety profile in humans. ***Methods***: A systematic literature search using PubMed and the Medline database was conducted between April and November 2020. ***Results***: Both preclinical (N=57) and clinical (N=18) studies emerged from our search. Preclinical data indicated a therapeutic value in terms of acute/chronic pain (N=23), morphine/ethanol withdrawal, and dependence (N=14), among other medical conditions (N=26). Clinical data included interventional studies (N=2) reporting reduced pain sensitivity, and observational studies (N=9) describing the association between kratom's chronic (daily/frequent) use and safety issues, in terms of health consequences (e.g., learning impairment, high cholesterol level, dependence/withdrawal). ***Conclusions***: Although the initial (pre)clinical evidence on kratom's therapeutic potential and its safety profile in humans is encouraging, further validation in large, controlled clinical trials is required.

New emerging trends of drug consumption among young individuals during the COVID-10 pandemic

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Introduction: According to the annual report of the Central Directorate for Drug Services, the COVID 19 pandemic has significantly affected the trends of substance misuse. Our study focuses on the seizures and consumption of substances in the Terni province in the centre of Italy. ***Methods***: A retrospective data analysis of drugs seized by the local police and further data collected by the local addiction services during the pandemic were carried out. ***Results***: In 2020-2021, compared to 2019, there was an increase in cocaine seizures (+806.82%) and hashish (+303.19%), with a decrease in all other substances; cocaine seizures had an escalation of over 800% (from 3.8 to 34.4 kilograms) considered the fourth largest increase in Italy whereas the amount of heroin seized declined from 6.7 kilos in 2019 to 1.82 in 2020. The Addiction Department of Terni, in the first half of 2021, registered an increase of 27% of subjects under the age of 25 assisted for addictions (63.3% males and 36.4% females). ***Conclusions***: These data show a significant increase of substance misuse among the individual under 25 that has occurred during the pandemic. We currently cooperating the with law enforcement agencies and drug services to monitor the phenomenon and design and implement adequate intervention strategies.

New psychoactive substance (NPS) trends in the United States

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Introduction: New psychoactive substances (NPS) have continually evolved since appearing in the United States in 2009. The timely dissemination of information outlining the NPS currently in the market provides useful information to the law enforcement and health communities. This presentation will illustrate NPS identifications and trends tracked by the Drug Enforcement Administration (DEA) Emerging Trends Program. ***Methods***: Data was collected for this analysis through a query of archived seizure and analysis information. The information targeted

in this query included the date and location of the seizure and substances identified during the chemical analysis performed by the eight DEA chemistry laboratories. These seizure details and analytical results are used to compile drug intelligence, detect the appearance of new drugs of abuse, and monitor drug trends. ***Results***: The most prevalent NPS identified in the United States fall within the categories of synthetic cannabinoids, cathinones, and opioids. There were three substances reported for the first time in the first half of CY 2021. Other chemical classes identified during the first half of CY 2021 include benzodiazepines, benzofurans, piperazines, and several other classes. ***Conclusions***: Due to the ever-changing nature of NPS, the criminal justice system is confronted with a unique set of challenges. Understanding the current trends and monitoring the emergence of NPS within the United States enables the health, forensic, enforcement, and legislative communities to be better prepared to fight the NPS epidemic.

Did the use of crystal meth increase with the easing of COVID-19 lockdown restrictions, leading to increased hospital admissions?

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Introduction: Methamphetamine, a derivative of amphetamine, is a central nervous stimulant first created in Japan in the 20th Century. Crystal methamphetamine ("crystal meth") is usually a more potent form of methamphetamine, and can be smoked, snorted, injected or orally ingested. Users of this drug experience an immediate "rush", with increased energy and euphoria. The psychiatric sequelae of crystal meth are well known, with psychotic symptoms such as hallucinations, persecutory delusions and ideas of reference developing in people who have recently taken the drug and that can last for several weeks. The use of this compound is not very common in the UK, however, certain groups of the population are at higher risk of crystal meth use, in particular people engaging in chemsex. ***Methods*****:** We audited the admissions for two North London psychiatric hospitals for the months of April and May, and reviewed whether crystal meth had been taken by patients prior to admission, and whether this had been a precipitating factor in their presentation. We compared admission data from 2019-2021, to observe any change in the number of presentations before the pandemic, during the height of London's lockdown, and as the lockdown restrictions began to ease. ***Results***: The data collected demonstrates a significant increase in the use of crystal meth with hospitalisation in 2021 compared to preceding years. Compared with 2020, there was a 150% increase in users of crystal meth presenting to emergency psychiatric services, leading to admission. ***Conclusions***: This new trend is worrying and it might be determined by different causes. Had accessibility to crystal meth increased following easing of lockdown restrictions? Did users of the drug have a reduced tolerance following a prolonged period of not accessing the drug, leading to a more serious psychiatric presentation? Why are there now more users of crystal meth compared to previous years; could this be explained by reduced availability of other commonly used illicit substances? Further studies are therefore necessary to confirm this new trend and to establish the causes.

Emerging novel designer benzodiazepine trafficking trends and risk to public health

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Introduction: In recent years, a growing number of "novel" "designer" benzodiazepines (BDZ) have appeared in illicit drug markets either alone or in combination with illicit drugs, as evidenced by law enforcement encounters. In addition, these substances are commonly advertised as "legal highs". At the same time, law enforcement encounters and prescriptions for the BDZ approved for medical use in the United States and law enforcement encounters associated with the same have decreased. Additionally, "designer BDZ" are increasingly identified in overdose deaths, calls to poison control centers and in cases of counterfeit drugs. ***Methods***: To identify the scope

of trafficking of pharmaceutical BDZ and designer BDZ, data from the National Forensic Laboratories Information System (NFLIS) database were queried and statistically analyzed. NFLIS is a DEA sponsored program that systematically collects drug identification results and associated information from drug cases analyzed by Federal, state, and local forensic laboratories. Additionally, to assess the trends in prescriptions for pharmaceutical BDZ, the IQVIA database was queried and analyzed. IQVIA identifies purchases by drug store retailers of ethical, ethical over-the-counter (OTC), and proprietary drug products projected to national U.S. levels as well as purchases by U.S. hospitals of ethical and proprietary drug products. Moreover, data regarding the involvement of BDZ in unintentional causes of death and multiple causes of death were obtained by querying the CDC WONDER database. Finally, data associated with the use of "designer BDZ" in counterfeiting was obtained from DEA's special testing laboratories and then further analyzed. ***Results***: While the diversion of the top five most often prescribed, pharmaceutical BDZ have decreased over the last 3 years, trafficking of the novel designer BDZ have increased markedly. Thus, data suggests that the decline in the illicit trafficking of medically approved BDZ is associated with the increase in non-controlled "novel BDZ". Data also suggests that when multiple causes of death exist, the involvement of BDZ in fatal cases are increasing. Additionally, the use of these non-controlled "designer BDZ" as counterfeit drugs in the illicit drug market has significantly increased in recent years. ***Conclusions***: The dramatic increase in trafficking, abuse, and fatalities associated with the novel designer BDZ in the United States has become a national public health concern in recent years. Therefore, control of BDZ with abuse potential are of significant public health importance. In response to this public safety issue, the international community has moved to recommend a number of designer BDZ for control.

Parallel Session V **Thursday November 18, 2021**

NPS Use Settings, Experience and Trends (II)

Impact of the COVID-19 pandemic on novel psychoactive substance (NPS) use, experience and risk awareness

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Introduction: Use of NPS has been strongly associated with influence of friends, social settings and sourcing via street and web-based platforms such as the darknet. Here we investigate whether the COVID-19 pandemic and lockdown measures have altered NPS use, preferences, substances resource and risk awareness internationally. ***Methods***: The Bristol Online Survey was in English and advertised on the drug forum Bluelight and social media Facebook pages and via University email between 8 August 2020 and 3 March 2021 (308 responses; 72 NPS users). This pharmacoepidemiologic study was evaluated using SPSS software (IBM SPSS Statistics version 27; MacOS Sierra 10.12.3). ***Results***: Specifically the main motivations for NPS use *prior* to the pandemic was to "experience something new and different" (55.1%), "get a good high" (47.8%) and "get help to cope with boredom and anxiety" (44.9%) whilst *during* the pandemic main motivations were to "cope with depression and anxiety" (53.8%), "get happier and more optimistic about life" (44.2%), and "get a good high" (36.5%). There remains a low perception of health risk associated with NPS use (75.7%). The preferred NPS of users *prior* and *during* Covid-19 was magic mushrooms psilocybin (61.8%; 49%) often in combination with alcohol (41.4%) and cannabis (37.1%). One out of two users stated that they changed the use of their favorite NPS during the pandemic; either increased NPS use due to anxiety (26.3%) and/ or because they had more available time (24.6%); or decreased due to their own choice to use less (29.8%). The Covid-19 pandemic and associated lockdown measures had an effect on settings of NPS use; *prior* the pandemic users mainly reported NPS use outdoors in nature with social gatherings (43.9%), in house parties (39.4%), in music festivals (37.9%) whilst during the pandemic reported use at home alone (48.1%), with household members at home (35.2%), outdoors in nature with other people (33.3%). Prior and during the pandemic there was no change in source(s) of NPS; the main sources remain friends/acquaintances and dealers (98%; 85.5%). One out of three users reported reduced availability of substances on the recreational drug market in their country when in contrast 30% reported no change. Respondents with underlying health conditions (asthma, mental health issues) vulnerable to Covid-19 reported significantly higher use of NPS ($p<0.05$) compared to respondents with no underlying health issues. Gender, age, living area, sexual orientation, place of living, educational background, smoking, alcohol consumption frequency and employment significantly affected ($p<0.05$) NPS use. Male respondents, residents of suburban and rural areas, smokers, respondents reporting frequent and high alcoholic consumption and respondents with low educational level represented the majority of NPS users as well as the employed, the unable to work and retired groups. Similarly, sexual orientation and religion significantly affected ($p<0.001$) NPS use. ***Conclusions***: Users' low perception of NPS safety profile especially in Covid-19 vulnerable with underlying health conditions makes it crucial to provide better education and information on NPS health risks and patterns during these emerging times. The fact that NPS use correlates with a lower level of education, indicates a need for enhanced statutory targeted prevention interventions in schools and colleges. Although the fact that many users during the pandemic have found an emotional healing, a source of happiness to cope with boredom, anxiety, and depression due to NPS use makes it important to investigate the therapeutic effects of these substances in clinical setting.

The detection, prevalence and modes of use of novel benzodiazepine-type drugs in prisons

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Introduction: New psychoactive substance (NPS) use in prisons is reported in many jurisdictions. In this presentation we highlight the use of novel benzodiazepine-type drugs in multiple drug formats in a prison context.

Methods: In situ and laboratory-based analysis methods including ion mobility spectrometry (IMS), gas-chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS-MS) were used to detect benzodiazepine-related substances in seized materials. ***Results***: Qualitative and quantitative analysis of >155 non-judicial/non-attributable seizures from Scottish was carried out. Etizolam, diazepam, alprazolam and flualprazolam were detected in tablets and powders. Etizolam was detected in marked and unmarked blotters (from September 2020); in infused bedding and clothing (from October 2020); and in infused greetings cards (from December 2020). Clonazolam, flubromazolam and flualprazolam have also recently been detected). We report a range of methods for consuming the infused materials. In two cases, the synthetic cannabinoid ADB-HEXINACA (>700 unmarked blotter tabs) and the novel synthetic opioid metonitazene (400 blotter tabs) in blotters visually similar to those containing etizolam were intercepted in mail sent to prisons. ***Conclusions***: We highlight the need to work in close partnership with prisons and other custodial establishments to rapidly identify changing patterns of drug use; to rapidly identify emerging drug threats; and to support the adoption of intelligence-led mitigation strategies.

Focus on over-the-counter drug abuse: a systematic review on the diversion of antihistamines, cough medicines and decongestants

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Introduction: Over the past 20 years or so, the drug misuse scenario has seen the emergence of both prescription-only and over-the-counter (OTC) medications being reported as ingested for recreational purposes. Among them, antihistamines, cough/cold medications, and decongestants, normally prescribed as OTC drugs, are reportedly being diverted and misused. While the current related knowledge is limited, the aim of this study was to examine the published clinical data on OTC misuse, focusing on antihistamines (e.g., diphenhydramine, promethazine, chlorpheniramine, and dimenhydrinate), dextromethorphan (DXM)- and codeine-based cough medicines, and the nasal decongestant pseudoephedrine, illustrating their psychotropic molecular mechanisms and related psychopathological effects. ***Methods***: A systematic literature review was carried out with the help of Scopus, Web of Science databases, and the related grey literature. For data gathering purposes, both the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) and PROSPERO guidelines were followed (PROSPERO identification code CRD42020209261). The assessment of risk of bias was made in accordance with the Cochrane risk of bias 2 (RoB 2) tool. ***Results***: After completion of the selection, eligibility, and screening phases, some 92 articles published up to December 2020 were here taken into consideration; case reports, surveys, and retrospective case series analyses were included. Findings were organized according to the specific OTC recorded. Most articles focused here on DXM (n = 54) and diphenhydramine (n = 12). When specified, dosages, route(s) of administration, toxicity symptoms (including both physical and psychiatric ones), and outcomes were here reported. OTC drugs were obtained by various means, including family and friends, multiple doctor prescriptions, illegal online pharmacies/shops, and theft/burglary from hospitals, residences, and pharmacies. DXM pills named “Snurf ” were also reported to have been acquired online and in having been marketed as a legal high. Overall, two main populations of OTC misusers were identified: (a) patients already suffering from a health condition and/or a psychiatric disorder who became dependent on their prescription/OTC drugs due to prolonged/high-dosage use, e.g. DXM-based cough mixtures started for sinusitis, cough, nasal congestion, and then continued for years at higher dosages; b) individuals, including substance abusers, not in treatment for a medical disorder or illness who may have started to misuse/abuse with OTC medications for recreational purposes. ***Conclusions***: Results from the systematic review showed that the OTC misusing issues are both widespread worldwide and popular; vulnerable categories included adolescents and young adults, although real prevalence figures remained unknown, due to a lack of appropriate monitoring systems. OTC recreational intake appeared to be associated with high/very high dosages; idiosyncratic routes of administration (e.g., snorting; IM; IV); and associated with ingestion of both licit

(e.g., alcohol, prescription opioids, benzodiazepines, other OTCs); and illicit (e.g., cannabis, cocaine, ketamine, etc.) drugs. Considering the potential, and at times serious, adverse effects associated with OTC misusing issues, healthcare professionals should be vigilant, and ad hoc preventative actions should be designed and implemented.

Concomitant findings in medico-legal death investigation and driving under the influence of drugs cases containing novel psychoactive substances

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Introduction: Identification of novel psychoactive substances (NPS) in medico-legal death investigation (MDI) and driving under the influence of drugs (DUID) cases is often one part of a larger testing strategy which may include alcohol, illicit drugs, and prescription and over the counter (OTC) medications. Trends in the types of drugs commonly encountered in cases which contain an NPS will be presented. ***Methods***: The laboratory information management system of a large reference laboratory was queried to identify cases submitted between January 1, 2016 and June 30, 2021 which contained an NPS and underwent a basic drug screen including benzodiazepines, barbiturates, opioids, cannabinoids, amphetamines, cocaine, and phencyclidine (PCP). Cases were categorized as MDI or DUID based on the client profile and type of testing that was requested. NPS were classified as opioids, mitragynine, benzodiazepines, synthetic cannabinoids, stimulants, hallucinogens, or dissociative and the frequency of concomitant findings was evaluated. ***Results***: Approximately 1300 DUID cases containing one or more NPS were identified. The most common class of NPS detected in DUID cases was benzodiazepines followed by synthetic cannabinoids, opioids, mitragynine and stimulants. Opioids were the most detected drug class found with NPS benzodiazepines and mitragynine while natural cannabinoids were most detected with synthetic cannabinoids. Approximately 250 NPS positive DUID cases contained a commonly prescribed or OTC medication. The most common combination was an NPS benzodiazepine in combination with buprenorphine. NPS analytes were identified in over 12500 MDI cases. In these cases, NPS Opioids were most common and were most detected in combination with fentanyl. Notably, nearly half of cases which contained an NPS opioid also contained a stimulant, primarily cocaine. Mitragynine was the next most frequent NPS followed by benzodiazepines and both were detected with opioids in most cases. Prescription and OTC medications were detected in over 60% of MDI cases the most common being an opioid antagonist drug such as Naloxone. After opioid antagonists, antidepressants and antihistamines are the most common classes of prescription and OTC drugs found in combination with all classes of NPS drugs. ***Conclusions***: Concomitant drugs use is common in both MDI and DUID cases which contain an NPS. Comprehensive drug testing is vital to understanding the scope and breadth of NPS use and to properly interpret findings.

Excessive exercise and IPED use among sport disciplines during the coronavirus disease 2019 pandemic

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Introduction: We examined the difference of tendency of addictive behaviors, such as excessive exercise and the usage of the image and performance enhancing drug (IPED) across 12 sport disciplines through the large sample set (N= 2,295). ***Methods***: The use of the IPED was assessed in conjunction with psychometric measures such as Exercise Addiction Inventory (EAI) and Appearance Anxiety Inventory (AAI). The participants were grouped into activity group (AG) and non-activity group (NAG) according to the presence or absence of their exercise habits. ***Results***: The frequency of IPED use was higher among AG (34.6%) than NAG (14.6%). The logistic regression analysis revealed that scores equal to or above cutoff points, in both the EAI and AAI, predicted the IPED use. Regarding the differences across the sport disciplines, those who were involved in practicing Weight Lifting and CrossFit were found to be more at risk of excessive exercising and more inclined to use the IPED. Conversely, those who were engaged in walking is low EAI as well as a low rate of IPED use. ***Conclusions***: These results may indicate

that excessive exercise is associated with the risk of cross-addiction with substance intake, particularly in disciplines that demand high-intensity functional training.

Parallel Session VI **Thursday November 18, 2021**

Detection, Identification, and Characterization

From isotonitazene to etonitazepyne: the continuing saga of newly emerging 'Nitazene'/2-benzylbenzimidazole opioids

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Introduction: Several 2-benzylbenzimidazole opioids (also referred to as 'nitazenes') have recently emerged on the illicit drug market. The most frequently encountered member, isotonitazene, has been identified in multiple fatalities since its appearance in 2019. Although isotonitazene was recently put under international control, many other analogues remain unregulated. Apart from a series of research articles exploring their analgesic potential in the 1950s-1960s, little is known about the harm potential of these increasingly encountered non-fentanyl opioids. Here, we discuss the *in vitro* μ-opioid receptor (MOR) activation potential of 12 nitazenes, including the most recently emerged 'closed-ring' analogues *N*-pyrrolidino ('etonitazepyne') and *N*-piperidinyl etonitazene ('etonitazepipne'), and 4 metabolites. ***Methods***: The *in vitro* MOR activation potential of twelve nitazenes and four metabolites was studied by means of a cell-based β-arrestin 2 (βarr2) recruitment assay. In short, activation of human MOR, fused to one subunit of a nanoluciferase enzyme, leads to recruitment of βarr2, fused to the complementing subunit. This results in the functional complementation of the enzyme, restoring its luciferase activity. Upon addition of a substrate, a bright bioluminescent signal is generated (NanoBiT®, Promega). From this, *in vitro* potency and efficacy values were calculated (the latter relative to hydromorphone). ***Results***: MOR activity determination confirmed that nitazenes are generally highly active, with potencies and efficacies of several analogues exceeding that of fentanyl. Particularly relevant is the unexpected very high potency of the *N*-desethyl-isotonitazene metabolite, rivalling the potency of etonitazene and exceeding that of isotonitazene itself. Our data further show that a number of variations to the general 2-benzylbenzimidazole core drastically impact MOR activity, enabling the assessment of structure-activity relationships. *N*-pyrrolidino and *N*-piperidinyl etonitazene have most recently emerged on the recreational drug market. With a potency comparable to that of etonitazene, the former (EC_{50} = 0.348 nM; E_{max} = 298%) is among the most potent (non-fentanyl) opioids described to date. *N*-piperidinyl etonitazene (EC_{50} = 1.60 nM; E_{max} = 252%) was around 5 times less potent than the pyrrolidino-analogue. Interestingly, our *in vitro* data correspond remarkably well with the rank order of antinociceptive potencies obtained in early studies in mice, and appear to be a better predictor of *in vivo* opioid activity than traditional binding assays. ***Conclusions***: As the presence of non-fentanyl opioids on the illicit drug market continues to rise, it will become increasingly important to rapidly identify and characterize new compounds as they emerge. Nitazenes are among the newest to appear and, given their high potential to activate MOR, their use may pose an imminent threat to any user.

Large-scale activity-based SCRA screening on patient plasma samples: CB1 bioassay supported by machine learning

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Introduction: Synthetic cannabinoid receptor agonists (SCRAs) are a prominent danger to public health. Emerging SCRAs are most often highly active at the CB1 cannabinoid receptor. This high activity imposes serious health threats, illustrated by intoxications with SCRAs presenting at emergency departments (ED's). The rapid emergence of novel analogs makes the detection of these new derivatives challenging. However, there is a strong need for continuous monitoring of these compounds to adapt legislations and ensure public health. The ED's of some hospitals, located in relevant areas regarding drug abuse, serve as some kind of 'sentinels', allowing to keep guard

on circulating, potentially highly dangerous SCRAs. An example is the ED of Guy's and St-Thomas' Hospital in central London, which is ideally positioned to keep track of the latest changes on the illicit drug market. ***Methods***: In the context of screening ED patient samples, the ideal assay is easy-to-perform and easily implementable. To speed up the work process and reduce the workload in the case of activity-based screening, we assessed whether artificial intelligence and machine learning could be of any help to the expert in deciding the eventual outcome of the screening assay. Following up on the success of a prior large-scale screening of serum samples for the presence of SCRA activity, we set out to screen a new large set (942 samples), with the following aims 1) assess the performance of the assay to using biological samples potentially containing newer circulating SCRAs, 2) exploration of a more structured way of manual scoring, and 3) exploration of computer-based scoring. ***Results***: Plasma samples, collected at the ED of Guy's and St-Thomas' Hospital in central London, were subjected to activity-based SCRA screening using a cell-based bioluminescence assay and to High Resolution Mass Spectrometry (HRMS)-based analysis for confirmation and identification. Both strategies were run independently and were performed blind-coded. Screening results from the bioassay (obtained through an improved scoring system by the expert) were compared with analytical (HRMS) results (considered as the 'gold standard'). The bioassay yielded a sensitivity of 94% and a specificity of 98%. The sensitivity obtained for a plasma sample volume of 250µL is in line with our earlier data, obtained on another sample set containing other SCRAs, where starting volumes of 500 or 100 µL were used. A positive correlation between sample volume and sensitivity was confirmed. The concluding specificity of 98% is in concordance with previously published results, which is very high for a broad screening assay. Sample volume does not seem to have a (pronounced) influence on this assay characteristic. The panel of identified SCRAs is largely distinct from the panel identified from April to December 2016, exemplifying the well-known phenomenon of market dynamics and, importantly, also underscoring the universal nature of activity-based screening. ***Conclusions***: A machine learning model was designed in order to automatically discriminate positive from negative samples. The model was trained on both the analytical outcome and the expert scoring to determine whether expert knowledge can be of added-value for training the predictive model. Two cross-validation settings were employed to evaluate the performance of the trained models and to assess the robustness of the machine learning approach. Depending on the desired sensitivity/specificity and the corresponding threshold applied within the model, we can conclude that machine learning is an adequate alternative for manual scoring by the expert (e.g. sensitivity of 94.6% and specificity of 94.6% at a 0.063 threshold). Automation of this scoring process results in significant time saving and reduction of the workload.

Designer benzodiazepines identified online: evaluation of their binding affinity for kappa, mu and delta opioid receptors through docking studies

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Introduction: Benzodiazepine (BZDs) are historically prescribed to heroin/opioids addicts to minimize withdrawal symptoms. Previous studies have reported that BZDs seem to enhance euphoric and reinforcing properties of opioids in opioids users. A direct effect of BZDs on opioid receptors has been postulated. The documented co-abuse of BZDs and opioids, that could lead to synergistic induction of severe respiratory depression, has become increasingly worrisome in the last decade, due mainly to the appearance on the market of the novel synthetic opioids (NSO) and designer benzodiazepines (DBZDs). The latter are mostly molecules whose activity/toxicology profiles are scarcely known. ***Methods***: A total of 101 DBDZs was previously identified online with a web crawler , NPS*finder®*. This study aims to evaluate their binding affinity (or lack thereof) towards the three opioids receptors, kappa, mu and delta, to assess if their mechanism of action could include the activation of the opioid's transmission and the synergic action on the latter. MOE® was used to perform the computational calculation. A pharmacophore map was generated for each of the 3 opioids receptors and used to filter the dataset of the 101 DBZDs identified. The filtered compounds were then docked into the crystallised 3D structures of the kappa (6B73), delta (6PT3) and mu(5C1M) receptors together with refences compound (i.e., selective and potent agonist

binders). Docking results reported binding affinity for a total of 9 (6B73), 13 (5C1M) and 10 (6PT3) DBZDs. ***Results/Conclusion***: Among these molecules Ciclotizolam, Fluloprazolam, JQ1, Ro 486791, Ro-488684 were found to show a better value of binding affinity, consistently across the three receptor subtypes. It may be inferred, that some DBZDs could have the potential to activate the opioids transmission. The latter could mediate and increase the anxiolytic, analgesic and addiction potential of these DBZDs, and aggravate the documented synergic effects between the two chemical classes of central nervous system depressant .

The pharmacoepidemiological value of para-Fluorofentanyl

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Introduction: Para-fluorofentanyl is a fluorinated analog of fentanyl with approximately one third the potency that can produce the same effects as other opioids. Para-fluorofentanyl was one of the many fentanyl analogs confirmed in drug chemistry and toxicology casework during the mid to late-2010s, but with low prevalence. Despite a February 2018 scheduling action by the DEA to temporarily control all fentanyl-related substances, the presence of para-fluorofentanyl has risen sharply between late 2020 and 2021. Para-fluorofentanyl is currently frequently detected with fentanyl in toxicological casework and drug chemistry analyses across the country, which raises the question of illicit sourcing, adulteration, and/or trafficking. ***Methods***: Toxicological cases reported between January 2019 and June 2021 was queried for reported para-fluorofentanyl in blood samples submitted from medicolegal investigations. Temporal changes, geographic trends, and additional drug findings were evaluated. ***Results***: In 2019, para-fluorofentanyl was detected in four cases (PA, NY, NJ, and NC). It was not reported again until Q4 2020, in 140 blood samples from 24 states; 5 states from different regions contributed > 10 confirmations each (AZ, MI, NY, PA, and TN). In Q1 and Q2 2021, there were 500 and 570 reported blood confirmations, respectively, from 40 different states, demonstrating a widespread increase. Reported blood concentrations (n=1214) averaged 6.6 ± 16 ng/mL (0.05-300 ng/mL). Fentanyl was detected in conjunction with para-fluorofentanyl (n=1049) 87% of the time when both analytes were covered under the scope of testing. Average fentanyl concentrations in the same cohort were 23 ± 37 ng/mL (0.31-530 ng/mL). In Q4 2020, 1% of fentanyl positive cases also detected para-fluorofentanyl; by June 2021 that percentage rose to approximately 19%. ***Conclusions***: The decline in fentanyl analog positivity in 2018 and the emergence of para-fluorofentanyl in 2020 can both be linked to legislative control of the drug(s) itself and the precursor(s) used during manufacturer, respectively. Due to its slightly lower potency, low concentrations in blood samples, and high concomitant detection with fentanyl, it is unlikely that para-fluorofentanyl is the primary driver of toxicity in these cases. For most cases encountered between 2020 and 2021, the value of para-fluorofentanyl is more pharmacoepidemiological, as detection and reporting of concomitant drugs, adulterants and by-products, can help serve as a “chemical signature” of illicit fentanyl manufacture. This data could assist investigators in tracking illicit fentanyl source and distribution into the United States and should be included in the scope of testing for both toxicological and seized drug casework.

Qualitative analysis of drug impregnated paper samples from England and Wales prisons in 2019 and 2020

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Introduction: Novel psychoactive substances (NPS) provide the same effects as classic drugs, such as cannabis, cocaine, and amphetamine. NPS are often smuggled into prisons by mail in the form of impregnated letters. The misuse of drugs in UK prisons is endemic, leading to violence, aggression, and disruptive behaviour among prisoners. The current research was conducted in order to develop a qualitative method to identify the variety of emerging novel psychoactive substances impregnated onto paper samples sent to prison inmates. The aim was to

help rapid detection and identification, enabling the scope of the problem to be established. **Methods**: From each piece of paper, from different locations, believed to be impregnated with drugs, approximately 1 cm square of paper was cut. Samples were placed into separate 1.5mL Eppendorf tubes with 1mL of 50% (v/v) methanol in LC-MS-grade water. Extracts were prepared from the samples by vortex-mixing (30 min). A mobile phase blank was injected between the analysis of each extract to check for carryover. Extracts were screened for NPS and other compounds using an Agilent Technologies 1290 Infinity II – 6545 Q-TOF LC/MS instrument with electrospray ionization in positive ion mode. It uses an Agilent Eclipse Plus C18 1.8 mm 2.1 x 100 mm column, maintained at 40^{o}C. Drug separation was performed over a total of 13 min using a simple linear gradient of water (A), and methanol (B), both contained 0.01 % (v/v) formic acid in 5 mmol/L ammonium formate, at a flow rate of 400 µL/min. Sample injection volume was 0.2 µL. ***Results***: A drug database which consists of more than 200 samples was created and utilized for the identification of unknown substances usually seen in forensic laboratories. 332 samples collected in 2019 from seven UK prisons and 563 samples collected in 2020 from four UK prisons were screened with this method. Synthetic cannabinoids were the most common drug category detected in prison letter samples in 2019. Miscellaneous and SCRAs are the most common drug category in 2020 samples. In Table 1, total is the total number for paper samples with their subsamples.

2019 Total		**Drug Types**	**2020 Total**	
Total	**Percent**		**Total**	**Percent**
0	0%	Anabolic steroids	0	0%
0	0%	Cannabis	0	0%
3	0%	Nicotine	35	2%
26	2%	Abused prescription drugs	37	3%
38	3%	Other medication	35	2%
564	51%	Synthetic cannabinoids	349	25%
75	7%	Miscellaneous	359	25%
45	4%	No drugs detected	256	18%
247	22%	Class A	309	22%
111	10%	Class B (excl. cannabis & SCRAs)	37	3%
1109	100%	Total	1417	100%

Table 1 *Percentages of the drug types in total in 2019 & 2020 in the UK prisons*

Conclusions: This research confirms that NPS being brought into UK prisons via drug impregnated letters posted to prisoners still remains a problem. With analytical confirmation of drug impregnated letters sent to prisoners, which include NPS, we have generated qualitative data to assist detection and identification information and have demonstrated that novel psychoactive substances are still entering UK prisons in this way.

Posters

Detection, Identification and Characterization

The development of methodologies for the identification and prediction of new synthetic cannabinoids

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Introduction: Synthetic cannabinoids are extremely popular within the prison system and cause problems for prisoners, law enforcement and health services. Synthetic cannabinoids are often soaked into paper then posted into prisons therefore, one of the aims of this research is to collaborate with Rapiscan Systems Ltd and local prisons to measure the effectiveness of trace detection methods for the identification of synthetic cannabinoids in letters using the ItemiserÂ®3Enhanced. ***Method***: To ensure compounds do not go undetected, samples that flag up on the ItemiserÂ® as unknown are analysed using Gas Chromatography-Mass Spectrometry, Nuclear Magnetic Resonance Spectroscopy and Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry to identify chemical characteristics which allow comparison to online reference spectra. ***Results***: To date, the method has identified seven compounds: ADB-FUBINACA, MMB-FUBINACA, 5F-ADB, 5F-MDMB-PICA, MMB-022, 4F-MDMB-BUTINACA and MDMB-4en-PINACA on documents entering the prison which were not already included on the ItemiserÂ® library. As a result, the libraries on prison instruments have been updated to ensure future detection of such compounds. ***Conclusion***: This has directly benefitted both the prison service and Rapiscan Systems Ltd and it is hoped that the continuation of this research could lead to a mutual exchange of information expanding to forensic providers and other research institutions.

NNL-3: a synthetic intermediate or a new class of hydroxybenzotriazole (HOBt) esters with cannabinoid receptor activity?

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Introduction: SCRAs containing ester linkers are intermediates en route to typical carboxamide SCRAs, but have been identified and marketed as NPS, exemplified by the suspected synthetic impurity NNL-3, a SCRA containing a hydroxybenzotriazole (HOBt) ester moiety. Similar HOBt esters can be formed and isolated during carboxamide SCRA synthesis. To explore this class of putative HOBt ester SCRAs, NNL-3 analogues were synthesized incorporating common SCRA scaffolds (indole, indazole, azaindole) and their binding affinity and functional activity at CB_1 and CB_2 was evaluated. ***Methods***: The biological activity of NNL-3 and 6 synthesized analogous HOBt ester SCRAs was evaluated at both CB_1 and CB_2, using earlier reported cell-based bio-assays developed to monitor β-arrestin2 recruitment to the CB_1 and CB_2 receptor. The assay relies on the NanoLuc Binary Technology® (Promega) which exploits functional complementation of a split nanoluciferase enzyme, of which the inactive subunits are either fused to the receptor (CB_1 or CB_2) and the intracellular protein βarr2. Receptor activation results in βarr2 recruitment, causing the 2 subunits to come in close proximity, resulting in restoration of the nanoluciferase activity and, following addition of substrate, luminescence. Activity of the compounds was also assessed using a fluorescence-based membrane potential assay which monitors Gβγ activation of inwardly-rectifying potassium channels, while binding affinity was evaluated using a competition [^{3}H]CP55,940 radioligand binding assay. ***Results***: The NanoBiT® assay highlighted 2 highly efficacious “super-agonist” compounds. Methylindole HOBt-2-Me-5F-PIC and its unsubstituted analog HOBt-5F-PIC exhibited efficacies at CB_1 of 724% and 831% respectively, in comparison to the reference compound CP-55,940, with potencies of 131 nM and 1.26 µM respectively. None of the compounds were scored as full agonists at CB_2, with HOBt-2-Me-5F-PIC being over 60-fold more potent than the other compounds at this receptor. These findings were confirmed by the radioligand binding assay, which revealed overall low affinity for both CB_1 and CB_2, except for HOBt-2-Me-5F-PIC and HOBt-5F-PIC. In line with the binding

and NanoBiT® data, the membrane potential assay scored HOBt-2-Me-5F-PIC and HOBt-5F-PIC as full agonists, while even at micromolar concentrations all other compounds showed negligible activity. Interestingly, addition of a methyl at the 2-position resulted in both increased potency at and affinity for both receptors. ***Conclusion***: NNL-3 as well as other 7-azaindole-bearing HOBt ester SCRAs showed only weak activity at CB_1 and CB_2. Methylindole and indole analogs, by contrast, were shown to be potent and efficacious, with substitution at the 2-position resulting in higher potency. This demonstrates that, while the marketed NNL-3 is unlikely to cause serious cannabinoid receptor-related toxicity, this cannot be concluded for the 2-methylindole HOBt ester (and its potential future analogues), with a potency and efficacy similar to that of SCRAs that have been involved in intoxications. However, one does have to keep in mind that the used assays can only determine *in vitro* activity, and cannot always accurately predict the *in vivo* effect as it will be subject to metabolic stability since esters may hydrolyze rapidly in the human body.

Identification of three arylcyclohexylamines (MXPr, MXiPr, and DMXE) in illegal products in Japan

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Introduction: A total of 2397 substances and two plants are controlled as “Designated Substances” in Japan as of August 2021 to prevent the abuse of new psychoactive substances (NPS). Although the distribution of these substances on the illegal drug market has decreased over the past six years, newly-emerged NPS are still being detected. Arylcyclohexylamines is a category of compounds to which the anesthetics phencyclidine (PCP) and ketamine belong. These compounds have been reported to act as antagonists of the NMDA receptor. An analog of ketamine, 2-(ethylamino)-2-(3-methoxyphenyl)-cyclohexanone (Methoxetamine, MXE), has been controlled as a narcotic in Japan and overdoses of MXE have been reported to cause health problems. In recent years, MXE derivatives have been detected in illegal products in Japan. In this study, we described the identification of three arylcyclohexylamines as designer drugs in illegal products. ***Methods***: Three powdery products were obtained in Japan between June 2020 and May 2021. Each product was extracted with methanol. The solution was filtered using a centrifugal filter and analyzed by LC-ESI-MS and GC-EI-MS. The accurate mass spectrum of the target compound was measured by LC-QTOF-MS. Identification of the compounds was performed using NMR (^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC and H-H COSY). ***Results***: From the illegal products, three compounds were detected by LC-MS and GC-MS. One compound, with a major ion peak on *m/z* 262 on the LC-MS total ion chromatogram, was identified as 2-(3-methoxyphenyl)-2-(propylamino)cyclohexan-1-one (Methoxpropamine, MXPr)**,** by comparing the data with the purchased authentic compound. The two unknown compounds (which showed putative protonated molecular ions at *m/z* 262 and 232 on the LC-MS total ion chromatograms, respectively) were analyzed by LC-QTOF-MS and NMR. They were identified as 2-(isopropylamino)-2-(3-methoxyphenyl)cyclohexan-1-one (Methoxisopropamine, MXiPr) and 2-(3-methoxyphenyl)-2-(propylamino)cyclohexan-1-one **(**Deoxymethoxetamine, DMXE). ***Conclusions***: In Japan, two arylcyclohexylamines are controlled as narcotics and 12 arylcyclohexylamines are listed as Designated Substances. It is reported that the value of IC_{50} of MXPr on NMDA-induced responses at the NMDA receptor is close to the result of MXE[1)]. For the regulation of the compounds identified in this study, the evaluation of their pharmacological properties is now in progress.

1) Irie T., Yamazaki D., Kikura-Hanajiri R., *Forensic Toxicology*, **39**, 474–480 (2021).

Detection of cannabinoids from fingernails by ATR-FTIR coupled with principle component analysis using chemometrics

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Introduction: The present study is an ongoing research project ,which offers a rapid and robust method to detect the presence of cannabinoids in fingernails. Arguably the nail can preserve more information on drug and its metabolites as compared to hair attributing to its thickness and presence of more keratin fibres along the length of the nail. Biomarkers present in the nail keratin fibers can be detected up to 3-6 months of drug consumption. Moreover, fingernail drug testing is a viable option where hair drug testing is not possible. ***Methods***: Sample collection: Fingernails from all ten fingers of 50 volunteer individuals with prior history of regular cannabis consumption after obtaining their consent were collected. Nails were clipped and collected in an aluminium foil tray. Sample treatment: All 10 nails from the individual were given an acetone wash and left at room temperature prior to ATR-FTIR analysis. Analysis of Collected samples with ATR-FTIR: The collected fingernail samples along with blank fingernail samples (Samples from individual with no prior history of cannabis use) were examined under the FTIR (Alpha-II, Bruker, Japan) model equipped with Platinum ATR featuring monolithic diamond crystal attachment. ***Results***: The preliminary results in the study demonstrated a sharp absorbance in the fingerprint region of IR spectra (1500-500 cm^{-1}) in individual with prior history of cannabis consumption and the spectra qualitatively correlates to the IR spectra of cannabinoids available from previously published literature online while the same was absent in blank samples. ***Conclusions***: The preliminary finding from the ATR-FTIR analysis demonstrates that it is possible to qualitatively detect cannabis use from the fingernails. The method is also proposed to be cross validated with HPLC equipped with ELSD, RID, and UV detector. PCA for whole spectra of all the individuals will be done and compared in order to comment upon the efficacy and accuracy of quantification. The method is rapid and easily executable with no prior sample treatment required and can generate reliable data to identify presence of cannabinoids in fingernails. The method can also differentiate between chronic drug users and people with no history of cannabis consumption.

Developing class-specific GC-MS methods for enhanced confirmation of NPS

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Introduction: With the increased prevalence of NPS and isomeric species, laboratories are looking for new approaches for compound confirmation. In this work, the development and implementation of GC-MS methods optimized for specific NPS classes is demonstrated. ***Methods***: Class-specific GC-MS methods were created using a six-step framework for development and evaluation. Development was completed using a multi-component test mixture and focused on maximizing chromatographic separation and understanding the interconnectivity of reproducibility and sensitivity. Once established, an expanded panel of compounds was analysed with the method to identify limitations in compound identification based on differences in retention times and mass spectral similarity. This was accomplished using a new, objective test that leverages replicate mass spectra. ***Results***: To date, methods have been created for synthetic cannabinoids, cathinones, and opioids. The methods have shown superior confirmation capabilities compared to general-purpose GC-MS methods, demonstrating enhanced sensitivity and increased capability for distinguishing spectrally similar compounds. The methods have been demonstrated to be suitable for street-samples. As new compounds are identified they are being added to the appropriate panel and any new limitations are being identified. ***Conclusions***: The use of class-specific GC-MS methods with known, objective, limitations in compound identification show potential for more confident confirmation of NPSs. This approach provides new capabilities to laboratories using their existing instrumentation.

Investigation of plant powder products distributed in Japan called "Acacia"

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Introduction: In recent years, plant powder products called "Acacia" have been distributed on the illegal drug market in Japan. These products contain a hallucinogenic tryptamine; *N, N*-dimethyltryptamine (DMT). A hot water extract from the product (called "Acacia tea") is usually taken together with a monoamine oxidase inhibitor (MAOI), as well as "Ayahuasca" which is used as a psychoactive brew in South America. In this study, the botanical origin of plant powder products sold as "Acacia" in Japan was identified by DNA analyses, and the amounts of tryptamines were analysed by LC-MS. In addition, "Acacia tea" was prepared from the products by the method described on the Internet, and the harmful effects of "Acacia tea" were evaluated. ***Methods***: Thirty-one plant powder products called "Acacia" in which DMT was detected, were obtained in Japan. Standard plant materials were collected from the tree *Acacia confusa*, which grows naturally in Okinawa Prefecture (the southern islands) in Japan. The standard plant materials were divided into 9 plant organs, such as leaves, branches, roots, etc. The "Acacia tea" was prepared with boiling water and citric acid from the plant powder products. DNA was extracted from the 31 products and the original plant species was identified by DNA sequence analyses. Two tryptamines, DMT and *N*-methyltryptamine (NMT) were analysed by LC-MS, in the products, in the standard plant materials and in the "Acacia tea". ***Results***: Each region of DNA amplicons obtained from the product was analysed. The phylogenetic analysis between the obtained DNA sequence and the public DNA database sequence suggested that the plant species used in the products was *Acacia confusa*. As a result of quantitative analyses by LC-MS, 11.4 to 49.9 (average 27.5) mg/g of DMT and 11.8 to 31.9 (average 20.8) mg/g of NMT were detected in the 31 products. In the standard plant materials, DMT and NMT were detected in all plant organs although their contents were different. The highest amounts of tryptamines were detected in the root bark, with DMT 37.2 to 61.2 mg/g and NMT 3.9 to 21.6 mg/g. In the branches and leaves, DMT was from 0.1 to 5.5 mg/g and NMT was from 0.4 to 14.9 mg/g. As for the "Acacia tea", the concentrations of DMT and NMT were 1.0 mg/mL and 0.9 mg/mL, respectively. ***Conclusions***: As a result of DNA analyses, it was concluded that the plant powder products "Acacia" distributed in Japan contained "*Acacia confusa*". *Acacia confusa* Merr. is a perennial tree native to South-East Asia in the family Leguminosae and is reported to contain DMT, mainly in its root bark. The highest amount of DMT in the 31 products was 49.9 mg/g and this result was not inconsistent with the amount of DMT in the root bark of the standard plant materials. It was reported that an oral intake of less than 50 mg of DMT could be effective when taken together with the MAOI. In the "Acacia tea", the concentration of DMT was 1.0 mg/mL and 50 mL of this solution may have some effect on humans. Some cases of poisoning caused by herbal solutions composed of Acacia tree bark and "Syrian rue seeds" (containing alkaloids with MAOI activity) were reported in Taiwan. To avoid health problems caused by plant products with psychoactive activity, we should continuously monitor the distribution of these products.

Advancements in detection of drugs of abuse and novel psychoactive substances for forensic investigations

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Introduction: In the field of forensics, drugs are one of the most recurrent and consequential evidence, and their accurate detection are crucially important. With the rise in technology, there are a vast number of instruments at the disposal by which even trace amounts of drug can be identified and quantified. ***Methods***: Literature for present study was collected from various online and open access databases like Google scholar, PubMed, WorldWideScience etc. using keywords relevant to the scope of the study. Research articles, review articles, scholarly articles in special edition journals, conference proceedings published in past 10-12 years were reviewed, analyzed and compiled in order to reach to a plausible conclusion. ***Results***: The analytical techniques can be used for the analysis of drugs belonging to stimulant, depressant, narcotic or hallucinogen classes as well as the novel

designer drugs or novel psychoactive substances (NPS) class. The detection of designer drugs are more crucial as there are new drugs synthesized regularly, which are structural analogues of illegal drugs and impart the same psychoactive effects, but are different enough so as to circumvent the law. The most preferred and adopted techniques include Gas chromatography (GC) and the Liquid Chromatography (LC or HPLC) coupled with the Mass Spectrophotometer (MS or MS/MS) as the detector, ATR-FTIR, Raman or NMR spectroscopy amongst many others. Accurate results with equivalent or better limits of detection were also obtained using techniques like Surface Enhanced Raman Scaterring (SERS) when compared with above mentioned conventional spectroscopic methods. ***Conclusions***: The review summarizes and gives a comparative account of different analytical instruments and techniques and their respective sample preparation time. It highlights the need of rapidity of detection without compromising on the limit of detection, research gaps, future scope of these techniques and the ones to come which will be used for the detection and quantification of drugs and their suitability in terms of detection limits.

Bromazolam: monitoring the increasing prevalence of an emergent designer benzodiazepine

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Introduction: Bromazolam is the brominated analog of alprazolam, a designer benzodiazepine (DBZD) that was first reported to the European Union (EU) Early Warning System in 2016 but has only recently started to gain market share in the United States (US). Bromazolam has comparable sedative and anxiolytic effects to other benzodiazepines. It follows the decline in flualprazolam positivity, its fluorinated counterpart, potentially in response to international control in November 2020. ***Methods***: Toxicological cases reported between January 2021 and August 2021 were queried for reported bromazolam in blood samples submitted from medicolegal investigations. Bromazolam is screened via liquid chromatography/time-of-flight mass spectrometry (LC-TOF/MS). Quantitative confirmation is achieved using liquid chromatography tandem mass spectrometry (LC-MS/MS) and the method of standard addition. ***Results***: In the first 8 months of 2021, bromazolam has been reported in 50 total cases from driving under the influence of drugs (DUID) and death investigations. The earliest collected sample was from December 2020. Three DUID cases reported concentrations of 170, 290, and 300 ng/mL. In the remaining 47 postmortem cases, the average and median concentrations were 789 ± 838 and 440 ng/mL, respectively (range 24-3800 ng/mL). Bromazolam has been reported in cases submitted from 18 different US states, as well as British Columbia and the United Kingdom. When evaluating the breakdown of the variety in designer benzodiazepines, etizolam is still the most prominent finding of this class, accounting for roughly 51% of DBZD detections when evaluating the first 6 months of 2021 screening data. Flualprazolam follows at 19% of detections, but that is a significant decrease from 33% in 2020. Clonazolam accounts for another 17% of detections, but mostly in the form of its metabolite 8-aminoclonazolam, and flubromazolam accounts for an additional 7% of detections. Bromazolam accounts for roughly 2% of detections, but month over month appears to be increasing. ***Conclusions***: Bromazolam is the newest designer benzodiazepine that should be monitored due to its increasing detection in medicolegal investigations. Bromazolam has central nervous system depressant (CNS) activity and therefore can cause impairment in operating a motor vehicle and/or may cause additive CNS depression with substances such as opioids that may result in overdoses. DBZD are frequently found in conjunction with novel synthetic opioids, both in drug chemistry (e.g., counterfeit pharmaceuticals, unknown powders) and toxicological findings. DBZD in general continue to rise in both prevalence and variety, as bromazolam is added to this growing class.

Early Warning Systems and Toxicovigilance

NPS as ingredients of ecstasy tablets

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Introduction: *Ecstasy*/MDMA is popular with clubs, discos and music festivals. In the world according to the EMCDDA, in 2017 in Europe the number of people aged 15-64 using this drug was 13.7 million (4% of this age group). The number of people who used this substance in the last year was 2.6 million (1%). The use of MDMA is more popular with young people. It is estimated that nearly 2% of young people (aged 15-34) used MDMA in 2017. Polish surveys conducted in 2008-2018 among school youth aged 18-19 showed that in 2018 the percentage of respondents who experimented with Ecstasy was the highest since 2013, compared to which there was a 2% increase, although it was still lower than compared to 2008 (5,5%). As for the percentage of people who used Ecstasy in the last 12 months, in 2018 it was lower than in 2016 and was at the level of 2% (in 2008 – 5,5%). On the other hand, the percentage of Polish students using Ecstasy in the last month was similar to the results from 2008 and was slightly above 1%. Low rates of Ecstasy use in 2010 and 2013 were directly related to the presence of 'legal highs' on the drug market in Poland, which were very popular at that time. Our study showed how the composition of *Ecstasy* tablets changed on the drug market in Poland in 2005–2019, in which, apart from MDMA, NPS was also detected. ***Methods***: The research material consisted of nearly 17000 tablets seized as Ecstasy and sent for examination to the Institute of Forensic Research in Krakow between 2005-2019. The qualitative analysis of the tablets was carried out by gas chromatography coupled with mass spectrometry (GC-MS). Gas chromatograph HP 6890N GC System coupled with the 5973 Network Mass Selective Detector with a quadrupole mass analyzer from Agilent Technologies (USA), were used in the study. Chromatographic separation of the tested compounds in the programmed temperature increase was carried out on the HP-5MS capillary column (30 m × 0.25 mm; 0.25 µm). The total analysis time was 31 minutes. The sample was dosed automatically in the splitless mode. The mass spectrometer operated in positive electron ionisation mode (EJ), and the energy beam was 70 eV. The acquisition was conducted in the scanning mode of the full mass range of m/z from 29 to 600 amu. Quantitative analysis was performed by ultra-high-performance liquid chromatography using a liquid chromatograph equipped with a spectrophotometric diode array detector (UPLC-PDA). The Shimadzu Nexera X2 chromatograph from Shimadzu (Japan) was used in the study. Chromatographic separation was performed on the Kinetex C18 column (50 mm × 2.10 mm; 1.7 µm) from Phenomenex in the reserved phase system, using gradient composition of the mobile phase. The mobile phase was a mixture of 0.01% phosphoric acid (V) in water (A, v/v) and acetonitrile (B, v/v). Spectra of substances were recorded in the spectral range from 200 to 400 nm. ***Results***: The most popular ingredient in the *Ecstasy* tablets tested was MDMA. The MDMA tablets were predominantly round (87% of the Ecstasy tablets with MDMA), with different colors and logos. The most popular logos found on these tablets were "Euro" (43%), "Jaguar" (5%), "Cock" (5%) and "Dolphin" (3%). 20% of the tablets tested had no logotypes. The mean weight of the MDMA tablets seized between 2005-2016 was 0.24 g (median 0.23 g, weight range 0.11 - 0.38 g). The mean weight of the MDMA tablets seized between 2017-2019 was 0.34 g (median 0.34 g, weight range 0.20 - 0.63 g). The mean content of MDMA in one tablet seized between 2005-2011 decreased from 90 to 20 mg. In 2013 *Ecstasy* tablets with a very high MDMA content (195 mg per tablet) have appeared on the market in the next two years, the MDMA content decreased again, which in 2015 was close to the values of 2006, 2009 and 2011 (50 mg per tablet). From 2016, the average MDMA content began to rise again, ranging from 90-140 mg. In 2017, we analyzed tablets containing 216 mg of MDMA. The *Ecstasy* tablets tested also contained NPS (20% of all examined tablets sold as *Ecstasy*), i.e. phenylethylamine derivatives (4-FA, 2CB-fly), piperazine (BZP, mCPP, TFMPP, DBZP and pFPP), tryptamine derivatives (5-MeO-DiPT and 5-MeO-MiPT), cathinone derivatives (DMC, pentedrone, MDPBP), aralkylamines (5-APB), arylcyclohexylamines (MXE, 3-MeO-PCP, PCP), piperidines (MXP), benzodiazepine derivatives (clonazolam), fentanyl derivatives (Fu-F) and methylphenidate derivatives (4F - MPH). In addition, caffeine, metoclopramide, piperonal, 1-PEA and theophylline were also detected. The popularity of these substances has changed over time. ***Conclusions***: Currently, the drug market is seeing a resurgence in the MDMA market in *Ecstasy* pills. New types of MDMA tablets are introduced to the market,

available in various colors and shapes. In recent years, studies have shown the availability of very strong *Ecstasy* tablets, containing over 140 mg of MDMA. Tablets sold as *Ecstasy* in addition to MDMA may contain completely different psychoactive substances (including NPS) belonging to different chemical groups or their dangerous combinations. Such a large variety of psychoactive substances in *Ecstasy* tablets is associated with a high risk for users unaware of their composition.

Monitoring New Psychoactive Substances

Correlation of self-reported psychiatric disorders, self-report scales and co-use of substances from an online survey among people who use Kratom

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Introduction: Kratom (Mitragyna speciosa) refers to a psychoactive plant preparation that has recently gained popularity across the globe as a novel recreational or therapeutic substance. A number of anonymous online surveys have been conducted in recent years on the demographics of kratom use. We incorporated standardized psychometric scales in a recently conducted online survey to help validate self-report and reduce bias, including self-reported diagnosed psychiatric conditions common among people who use kratom (PWUK), such as depressive and anxiety disorders, with other outcome measures. ***Methods:*** An anonymous online survey was distributed using Qualtrics via social media platforms (Facebook, LinkedIn, Reddit), kratom advocacy websites, and kratom distributors from July 2019 to 2020. The survey contained the Euro-QOL-5D-5L, ASRS-v1.1, PC-PTSD-5, and SCL-90 self-report scales. Participants were assessed for their self-ported diagnosed health conditions, kratom use patterns, and previous or concurrent use of other substances, among other items. A total of 4,945 valid responses were included in the analysis. Bivariate and interval analysis were conducted; statistical significance was set at $p \leq 0.05$. ***Results:*** Of those who self-reported a diagnosed psychiatric condition, about one-third of the participants met each respective scale's clinically relevant criteria threshold for attention-deficit/hyperactivity disorder (ADHD) (39.6%), post-traumatic stress disorder (PTSD) (34.6%), anxiety (39.2%), and depression (37.0%). There was a high correlation between poly-substance use with kratom and a self-reported prior substance use disorder treatment (80.2%). Kratom amount/dose differed significantly for those meeting PTSD diagnostic criteria ($p<0.01$), while kratom use frequency was significant for those meeting anxiety disorder criteria ($p<0.01$). More young respondents between the ages of 21-40 met the diagnostic criteria for ADHD and PTSD. ADHD and PTSD were more commonly diagnosed in female respondents ($p<0.01$) whereas males self-reported more frequently ADHD diagnosis. ***Conclusions:*** The correlation between self-reported diagnosed psychiatric disorders and validated psychometric scales indicates a more complex picture of biased self-reporting and the dimension of diagnosed psychiatric disorders. Among PWUK in particular, psychiatric disorders are highly prevalent and correlations between kratom dosing, frequency of use, and severity of anxiety and other disorders are common. This complexity warrants further research beyond this preliminary investigation.

Synthetic psychoactive substances advertised on one cryptomarket over a one-year period

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Introduction: The pursuit of fun among "clubbers" and disco-goers often involves the use of drugs and use of Novel Psychoactive Substances (NPS) among them. However, whether this substance use can relevantly impact mental health remains debated. ***Methods***: To address this issue, we collected data during four consecutive years in dedicated nursing units inside all the nightclubs of Ibiza, in emergency hospital rooms at the Can Misses Hospital and inside the psychiatric ward. ***Results***: A total of 10,163 subjects required medical assistance inside discos in the

medical-nursing units and were included in the analysis. The present work describes trends in substance use, risk for development of long-term psychiatric sequelae and suicidal behaviors among disco-goers in Ibiza.

A pandemic during the pandemic? Impact of COVID-19 on cannabis use, patterns and settings for drugs use support

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Introduction: The Covid-19 pandemic has significantly affected people's lives globally. Lockdown measures and social distancing have a negative impact on people's mental health state. To help cope with mental health issues, such as anxiety and depression, some people use psychoactive substances. Here we investigate whether the Covid-19 pandemic and lockdown measures have altered drug use, and specifically, cannabis use. Additionally, we examined how drug users perceived drug support system during the pandemic and their drug use preferences/motivations. ***Methods***: The survey was conducted by Drugs and Me (https://www.drugsand.me/en/) using the SurveyMonkey platform. It was in English and advertised on social media during April 2020, at the time when most countries around the world implemented social restrictions. This pharmacoepidemiologic study was evaluated using SPSS software (IBM SPSS Statistics version 27; MacOS Sierra 10.12.3). Chi-square test was used to assess categorical changes. The significance level was set to *$p<0.05$ after a Bonferroni correction. ***Results***: 1,615 out of 2,450 respondents reported psychoactive substance use. 1,315 (81.4% of those reported psychoactive substance use) claimed to have used cannabis since the pandemic had started. Region of residency and age significantly predicted cannabis use ($p<0.05$). Most of the cannabis users reported daily use, an increase of cannabis use during the outbreak and their intention to not stop cannabis use. Those that reported greater alcohol consumption during the pandemic also increased their cannabis use ($p<0.001$) and tended to combine cannabis with alcohol ($p<0.001$). Nicotine users in majority were habitual daily cannabis users ($p<0.001$). Increased nicotine smoking correlated with an increase of cannabis use during the outbreak ($p<0.001$) and a more habitual cannabis use ($p=0.006$).

Category	Number responses (%)
Total responses **''Cannabis users''**	1315 (100%)
Gender (p= 0.086)	
· Male	877 (66.7%)
· Female	394 (30%)
· Other *	44 (3.3%)
Age (p<0.001)	
· < 18	113 (8.6%)
· 18 – 25	707 (53.7%)
· 26 – 40	398 (30.3%)
· 41 – 60	89 (6.8%)
· 61 +	8 (0.6%)
Countries (p<0.05)	
· UK	394 (30%)
· USA & Canada	396 (30.1%)
· Australia & New Zealand	66 (5%)
· Europe	391 (29.7%)
· Others**	68 (5.2%)
Employment (p=0.813)	
· Student	573 (43.6%)

· Employed	473 (36%)
· Self employed	101 (7.7%)
· Unemployed	155 (11.8%)
· Retired	13 (0.9%)
Alcohol use (p=0.55)	
· Yes	911 (69.3%)
· No	404 (30.7%)
Nicotine use (p=0.092)	
· Yes	715 (54.4%)
· No	600 (45.6%)
Main reasons for drugs use: (multiple response question)	
· To have fun	947 (72%)
· Have a relaxing night out	908 (69%)
Support regarding drugs use: (multiple response question)	
· Before the pandemic ''not efficient''	595 (61.4%)
· During the pandemic ''not efficient''	839 (86.6%)
· Not seeking for drugs consultation	822 (84.8%)
Cannabis dependent	446 (33.9%)
Intention to stop cannabis use	109 (8.3%)
Frequency of cannabis use	
· Daily/ every other day	811 (61.7%)
· 2 or 3 times a week	227 (17.3%)
Change of cannabis use during the pandemic	
· Increase	466 (35.4%)
· Great increase	214 (16.3%)
Withdrawal symptoms	180 (13.7%)

*** Others: no binary, not saying**

**** Others: Africa, Central & South America, Middle East & Asia, Russia**

Significant difference *P < .05, χ2 test

Conclusion: The Covid-19 outbreak associated social isolation measures have raised concerns regarding substance use. Due to a decline in people's mental health as a result of social isolation, lack of services for drug use support, and limited drug education, both public and scientific spheres should be concerned about significant changes in substance use. This study shows that variables such as age and polydrug use are relevant to identify those at risk of increasing their substance use and should guide harm reduction efforts. There is a need for organised strategies aiming for better drugs education and support through public health sectors, universities, government including an update of drugs policies signposting to drug education and harm reduction support.

The shifting synthetic cannabinoid market in New Zealand, August 2020 to March 2021

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Introduction: Since 2017 and in response to the apparent increased harm associated with the use of synthetic cannabinoid preparations ("synthetic cannabis") in New Zealand (NZ), the Institute of Environmental Science and

Research (ESR) has carried out regular surveys to determine the types and concentrations of synthetic cannabinoids available. Here, the results of the most recent survey of synthetic cannabinoids present in NZ law enforcement seizures between August 2020 and March 2021 will be presented, together with a comparison of previous survey results. ***Methods***: Between August 2020 and March 2021, NZ law enforcement seizures of "synthetic cannabis" plant material were submitted to the ESR Laboratory for analysis. These samples were screened for the presence and concentration of synthetic cannabinoids using gas chromatography-mass spectrometry (GC-MS). ***Results***: Between August 2020 and March 2021, there were a total of ten different synthetic cannabinoids detected in domestic seizures of "synthetic cannabis" plant material. Six of the substances detected were new to NZ. The three most prevalent synthetic cannabinoids were MDMB-4en-PINACA, AB-FUBINACA and 5F-MDMB-PICA. The two most popular synthetic cannabinoids AMB-FUBINACA and 5F-ADB, detected in earlier surveys, were virtually non-existent compared to earlier trends in NZ. Along with a change in the type of synthetic cannabinoids detected, a novel preparation was also observed, with AB-FUBINACA impregnated onto tobacco plant material, instead of the usual damiana-type preparations. ***Conclusion***: Earlier legislative changes in New Zealand, such as the listing of AMB-FUBINACA and 5F-ADB as Class A controlled drugs, have shown continued effects in terms of the type of synthetic cannabinoids detected in the survey. The market in NZ has experienced a lag period compared to the rest of the world prior to the detection and now prevalence of MDMB-4en-PINACA. The novel preparation of synthetic cannabinoids on tobacco may lead to difficulties for enforcement agencies.

Media depiction of Ketum (Mitragyna speciosa) issues in mainstream Malay-Language newspapers in Malaysia

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Introduction: People who use ketum (Mitragyna speciosa) (PWUK) continue to be penalized because its use is illegal in Malaysia. On the other hand, self-reports from users around the region indicate a variety of health benefits from its use. This study examines how popular Malay-language newspapers have reported on ketum-related issues over the past two years. Since ketum use is widespread among Malays in rural communities, and a large proportion of policymakers are Malays, it is reasonable to assume that Malay newspapers influence both consumers and policymakers regarding the utility (or otherwise) of ketum. ***Methods***: A content analysis was carried out using words such as "ketum", "ketum seizure" and "ketum distribution" to retrieve media reports about ketum from three popular Malay-language newspaper outlets that had the widest coverage on this topic (Harian Metro, Sinar Harian and Berita Harian). Articles were restricted to those that appeared in the last sixteen months (March 2020 to July 2021) to capture current reporting on the topic. The narrations were then coded by two trained research assistants. ***Results***: All three media outlets reported extensively on ketum seizures by the Malaysian Border Security Agency and Royal Malaysia Police, the smuggling of ketum into Thailand for trafficking, the forms of ketum seized or smuggled, the street value of ketum recovered, and the laws under which those caught for these activities were detained. Media reports also indicated that the smuggling and distribution of ketum were rampant in the northern states of Kedah, Perlis and Kelantan that were close to the Thai border. ***Conclusion***: Mainstream Malay newspapers have focused primarily on the law enforcement perspective of ketum. In doing so, they have cast ketum in poor light, associated it with deviant behaviour and will motivate continuing law enforcement efforts to suppress it. Absent from the newspapers were perspectives on the potential utility of ketum emerging from academic studies including its usefulness in aiding withdrawal from heroin. The newspapers played no role in kindling informed debates about the pros and cons of a widely available, widely consumed and potentially useful plant.

NPS Pharmacology and Toxicology

Amphetamine-like stimulants induce oxidative stress and DNA damage in human neuroblastoma SH-SY5Y cell line

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Introduction: Amphetamine-like stimulants (ATS) are widely abused substances that impair dopaminergic and serotonergic functions. The mechanisms of their toxicity in human neuronal cells are not clear yet, but they may involve the formation of reactive oxygen species. The aim of this study was to determine the induction of oxidative stress parameters and the levels of DNA instability in human neuroblastoma SH-SY5Y cells treated with selected concentrations of four different ATS during 24 hours. ***Methods***: SH-SY5Y cells were treated with ATS at concentrations that did not decrease viability below 75 %. A new psychoactive substance mephedrone was tested at 6.25 μM, while so-called classical ATS were applied as follows: 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) at 0.625 μM, methamphetamine at 0.625 μM, and amphetamine at 3.13 μM. After the treatment, cells were trypsinized, washed in phosphate-buffered saline, resuspended in complete medium and prepared for further analysis. The ATS-toxicity was evaluated using markers of DNA damage and oxidative stress. Lipid peroxidation level (malondialdehyde (MDA)), reactive oxygen species (ROS) production, glutathione (GSH) levels, and activities of antioxidant enzymes (catalase, glutathione peroxidase and superoxide dismutase) were measured as biomarkers of oxidative stress, while primary DNA damage was determined using alkaline comet assay. ***Results***: When compared to controls, cells treated with methamphetamine had a significant increase in MDA, cells treated with mephedrone had a significant increase in ROS, GSH and GPx, whereas cells treated with amphetamine had a significant increase in ROS, and a significant decrease in GSH. The range of DNA damage measured with the comet assay after treatment (based on the median values obtained for tail intensity) was: amphetamine > methamphetamine > mephedrone > MDMA. ***Conclusions***: Significant induction of oxidative stress parameters and increased level of DNA damage detected at cell level call for further studies to clarify the toxicological risks associated with ATS consumption.

Not just the mental issues: NPS implications in cardiology

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Introduction: The last decade has seen a conspicuous growth in the number of psychoactive substances, predominantly appeared to be beyond international control. The toxic effects of such new formulas embrace a wide range of symptoms, where mental disparities prevail in an acute phase of NPS intoxications. Meanwhile the high mortality are more likely to be related to somatic symptoms, especially cardiac abnormalities. The aim of the study was to investigate the laboratorial and instrumental indicators in routine clinical tests associated with NPS abuse and presented beyond acute NPS intoxication. ***Methods***: We conducted cross-sectional comparative study analysing the medical documentation of 566 patients with drug addictions: 191 NPS users and 365 non-NPS users. Among other variables we estimated liver transaminase levels, hematocrit, and hemoglobin, and ECG parameters. ***Results***: NPS patients were presented with significantly higher levels of haematocrit: 0.42±0.4 L/L versus 0.39±0.5 L/L; and haemoglobin: 142±14.5 g/L versus 132.9±18.1 g/L. Abnormal repolarization and left ventricular hypertrophy were also more prevalent among NPS patients: 21,5% versus 11.2% and 21.5% versus 14.9% respectively. In regression analysis, the duration of drug use (OR=1.07, $p<0.001$) and NPS use (OR 3.11, $p<0.001$) were the factors associated with left ventricular hypertrophy. For the abnormal repolarization those factors were as follows: the duration of drug use (OR=1.04, $p=0.04$) NPS use (OR 2.63, $p=0.002$) and male gender (OR 2.49, $p=0.015$). ***Conclusions***: Our findings warrant the need of further investigation to obtain insights into cardiological side effects of NPS considering the capabilities of routine clinical tests.

Cyclopropylfentanyl prevalence and analysis, 2017–2020

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Introduction: In June 2017, cyclopropylfentanyl made headlines when the Georgia Bureau of Investigation Crime Laboratory identified its presence in counterfeit pills, along with U-47700. Since then, cyclopropylfentanyl has been linked to numerous fatalities across the United States and around the world. It is currently a Schedule I drug in the United States with no accepted medical use. ***Methods***: Antemortem driving under the influence of drugs (DUID) and postmortem specimens confirmed positive for cyclopropylfentanyl by NMS Labs from 2017 through 2020 were reviewed. ***Results***: Since 2017, cyclopropylfentanyl has been identified in approximately 912 specimens at NMS labs: 652 quantitative and 260 qualitative. Identifications increased through the fourth quarter of 2017 but declined throughout 2018 with only 34 confirmations occurring in 2019. Twelve DUID cases were positive for cyclopropylfentanyl between October 2017 and June 2018 with most of these cases occurring in Pennsylvania. Common drug combinations in this population included cyclopropylfentanyl with cocaine, fentanyl, marijuana, and morphine. Approximately 634 postmortem cases were quantitated for cyclopropylfentanyl in blood between July 2017 and December 2020. While identifications in postmortem cases occurred across the United States, the majority were in Illinois. Common drug combinations in postmortem cases included cyclopropylfentanyl with fentanyl, marijuana, and cocaine. Drug concentrations for DUID and postmortem cases are summarized below in Table 1.

Case Type	N	Mean (±SD) (ng/mL)	Median (ng/mL)	Concentration Range (ng/mL)
DUID	12	3.9 ± 6.8	1.4	0.09 - 26
Postmortem Blood	634	11 ± 24	6.0	0.05 - 330
Vitreous Fluid	8	6.2 ± 5.4	5.9	0.24 - 17
Liver*	8	191 ± 131	175	4.5 - 380

Table 1: The total number of reported quantitative cyclopropylfentanyl positives (N), mean±SD, median, and concentration range.
***units are ng/g**

Seven postmortem cases confirmed cyclopropylfentanyl in peripheral blood, vitreous fluid, and liver. These concentrations are summarized in Table 2 below. Based on these results, vitreous fluid and liver are acceptable alternative specimens for cyclopropylfentanyl testing in cases where postmortem blood may not be available.

Case Number	Peripheral Blood (ng/mL)	Vitreous Fluid (ng/mL)	Liver (ng/g)
1	7.9	5.6	170
2	2.6	1.3	19
3	24	12	350
4	57	6.1	380
5	43	17	290
6	17	6.7	180
7	0.32	0.24	4.5

Table 2: Cyclopropylfentanyl concentrations in peripheral blood, vitreous fluid, and liver in seven postmortem cases.

Conclusions: The fentalog cyclopropylfentanyl gained popularity in 2017 but confirmations declined throughout 2018 and into 2020. During this time, it was identified by NMS Labs approximately 912 times. Mean and median drug blood concentrations were higher in postmortem cases compared to DUID cases, as expected. Identifications occurred across the United States with most occurring in Pennsylvania and Illinois for DUID and postmortem cases,

respectively. Liver and vitreous fluid are acceptable alternate matrices for cyclopropylfentanyl testing in cases where postmortem blood may not be available.

Safety profile and effects of mitragynine in healthy volunteers

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Introduction: Mitragynine (MG) is one of the main psychoactive components in kratom. Preclinical studies suggested potential therapeutic effects of MG, including antinociception. Clinical evidence on its safety and therapeutic properties is scarce. Therefore, this study aims to investigate MG safety and its effects in healthy volunteers. ***Methods***: A placebo-controlled, single-blind, within-subjects study started in April 2021 and is currently running (N=8; mean age: 24.1). Treatments are three single oral doses of MG (5, 10, and 20 mg) and placebo which are administered on separate test days and in an incremental dosing scheme, given the focus on safety. Safety parameters, experience questionnaires, cognitive tasks, and pharmacokinetics were assessed repeatedly before and after dosing. ***Results***: On the overall number of attentional lapses in the Psychomotor Vigilance Task; MG-placebo contrasts showed this effect was attributable to the 5 mg dose causing an increase in attention compared to placebo. Analyses did not show MG effects on other cognitive tests or questionnaires, or vital signs and laboratory safety, compared to placebo. Pharmacokinetic analyses showed a dose-dependent concentration in blood of both MG and its active metabolite 7-hydroxy-Mitragynine (7-OH-MG). ***Conclusions***: It can be put with caution that MG is safe at the three administered doses, future analyses on the complete dataset are needed to draw definite conclusions on the safety and effect profile of MG.

Alprazolam-related deaths in Scotland

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Introduction: There is no prescribing of alprazolam in UK primary care. However, there has been a rapid increase in demand for illicit supply and consumption. Simultaneously, there has been an increase in alprazolam-related fatalities. This study examined patterns in such mortality in Scotland. ***Methods***: Anonymised case-level data were obtained from the National Records of Scotland for drug poisoning deaths registered between 2015 and 2020. Records were extracted where alprazolam was mentioned in the 'cause of death' and/or the list of substances considered by the pathologist to have caused or contributed to death. Characteristics of decedents, cause of death and implicated substances were examined using IBM® SPSS Statistics (version 26) and Microsoft® Excel® 365. ***Findings***: 366 cases were identified, peaking in 2018. Four-fifths (77.1 %) were male; mean age was 39.0 (SD ±12.6) years. Underlying cause of death in most cases was accidental poisoning, principally involving opiates/opioids (77.9%) and sedatives, hypnotics and similar substances (15.0%). Mean number of substances involved in deaths was 4.77 (SD ±1.821); in only two cases was alprazolam the sole substance involved. Main drug groups implicated were: opiates/opioids (94.8%); other benzodiazepines (67.2%); gabapentinoids (42.9%); stimulants (30.1%); antidepressants (15.0%). Two-thirds (64.2%) involved both other benzodiazepines and opiates/opioids. ***Conclusions***: Recreational alprazolam use is still rising. More than 5% of Scottish drug poisoning deaths involve the substance. Monitoring such deaths should continue. Those prescribed alprazolam or considering acquiring illicit supplies for recreational use should be aware of the dangers of using it with other drugs, especially opiates/opioids and other benzodiazepines.

Alternative matrices in forensic testing: comparative evaluation of different analytical protocols on dried spots

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Introduction: Dried blood spots are a form of biosamples in which capillary blood by finger or arm pricking is applied onto marked circles on cellulose paper. This technology has been successfully adopted for neonatal screening since 1963, and it is now also considered in anti-doping analysis. In the last two decades the use of capillary blood, plasma, and urine applied to an inert support as alternative biological matrices has been progressively extending, now covering many different applications, including forensic analysis. Indeed, the use of these matrices possesses numerous advantages over traditional blood and urine samples: simplified and minimally invasive sample collection procedures, enhanced compound stability, no requirement for controlled temperature transport and storage, with a remarkable reduction of the overall costs. ***Results***: Here we report preliminary data obtained by evaluating the potential of different microsampling devices and extraction protocols to determine drugs of abuse in dried matrices (capillary blood, plasma, and urine).

Safe testing in the COVID age: selective inactivation of coronaviruses in urine forensic samples by intermittent microwave irradiation

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Introduction: We present a method to drastically reduce the viral load of human urine samples collected in the framework of forensic toxicology testing, with special reference to doping analysis. ***Methods***: The method was specifically developed to reduce the viral load of SARS-CoV-2, but showed to be effective also on other coronaviruses. The method was tested on urine samples fortified with SARS-CoV-2 (3 x 105 RNA copies), and feline coronavirus (FCoV-II, 4.2 x 108 RNA copies). To verify whether the microwave treatment could alter the integrity of the sample, aliquots from the same batch of samples, free of viruses, were fortified with target compounds routinely screened for by the antidoping laboratories, representative of the different classes of prohibited substances, and analyzed after the microwave treatment. Forensic DNA analysis was also carried out before and after the microwave treatment. ***Results***: Optimal treatment conditions were the following: irradiation in three consecutive irradiation steps, (20 s irradiation + 30 s rest) at 1200 W emitted power, reaching a steady state temperature of 65 °C in the first 20 s and maintaining it along the entire process. In the above conditions SARSCoV-2, and FCoV-II loads were reduced by 1.96 (99%) and 4.8 (99.99%) Log, respectively. At the same time, all the representative target compounds (low molecular weight xenobiotics and their main metabolites, small and large peptides, including recombinant insulins, IGF-1, recombinant human erythropoietin and other erythropoiesis stimulating agents, luteinizing hormone and human chorionic gonadotropin, markers of the urinary steroid profile) were unaffected by the treatment, resulting still detectable after microwave irradiation and without any significant change in their measured concentration. DNA extraction and identification was also unaffected by the treatment. ***Conclusion***: The method here presented ensures the safe and effective anti-doping analysis of urine samples also in the pandemic days.

<u>NPS Use, Settings, and Trends</u>

Novel psychotropic substances in sports: forensic review

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Introduction: Use of conventional psychoactive substances has been observed in sports since time immemorial. Novel Psychotropic Substances (NPS) are a major threat to the ethical standards of sports and other relevant areas.

Issues with regard to NPS could be easy access to raw material for synthesis, worldwide distribution and difficulty in identifying the NPS with lack of any reference materials reported already. A common platform of information sharing may mellow down the menace associated with NPS and doping of the same in sports. ***Methods***: A systematic literature review based on defined criteria and protocol would be carried out. Thorough analysis of all the aspects of the given topic would be carried out. Specific research questions with regard to investigation and detection of NPS would be covered in the study. Synthesizing the whole study with reference to current times. ***Results***: Performance and Image Enhancing Drugs (PIEDs) are many times used without the reference of any medical need. Lack of understanding of psycho-physiological changes of NPS and proper guidelines is the immediate need. Proper understanding of cases of NPS in sports needs to be developed with regard to specified guidelines. ***Conclusions***: Utility of IT tools can be an immediate solution with relevant databases in the NPS found in the cases of sports forensics. Merging the details of clinical information with the investigational outline time to time would always be inevitable. Combating the problem of under-reporting may solve the issues with regard to identification and investigation of such cases.

The use patterns of novel psychedelics: experiential and contextual fingerprints of substituted phenethylamines, tryptamines and lysergamides

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Introduction: Novel psychedelics (NPs) are an expanding set of compounds, presenting new challenges for drug policy and opportunities for clinical research. Unlike their classical derivatives, little is known regarding their use profiles nor their subjective effects. ***Methods***: A two-part survey was employed. We investigated the prevalence of novel phenethylamines, tryptamine and lysergamides in NP users (N = 1180), contrasting the type and incidence of adverse events (AEs) using a set of logistic regressions. Honing in on 2-4-Bromo-2,5-dimethoxyphenyl)ethanamine (2C-B) (48.6%), 1-propionyl-lysergic acid diethylamide (1P-LSD) (34.2%) and 4-Acetoxy-N,N-dimethyltryptamine (4-AcO-DMT) (23.1%), we cross-examined their phenomenology using a gradient boosting (XGBoost) supervised classifier. ***Results***: Novel phenethylamines had the highest prevalence of use (61.5%) seconded by tryptamines (43.8%) and lysergamides (42.9%). Usage patterns were identified for 32 different compounds, demonstrating variable dosages, durations, and a common oral route of administration. Compared to phenethylamines, the odds for tryptamines and lysergamides users were significantly less for overall physical AEs. No significant differences in overall psychological AEs were found. Model sensitivity (50.0%) and specificity (60.0%) for 2C-B ranked lowest, whereas 4-AcO-DMT was most frequently discriminated. ***Conclusions***: Novel psychedelics may hold distinct adverse event rates and phenomenology, the latter however potentially clouded by the subjective nature of these experiences. Further targeted research is needed.

Prevention, Harm Reduction, Treatment and Clinical Management

Incorporation of novel psychoactive substance testing into routine outpatient healthcare toxicology testing menus

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Introduction: Urine drug testing is one strategy utilized to help ensure individuals are adherent to a prescribed drug regimen and identify substances, which may adversely impact patient care in the pain management, psychiatric, and substance misuse specialties. In pain management settings, patients may feign symptoms to continue receiving prescription opioids, which may be later diverted, or they may supplement with novel psychoactive substances (NPS) which can increase the risk of adverse events. In psychiatric settings, patients may not have the ability to adhere to complex treatment regimens, depending on their condition. Individuals with

substance use disorder may not adhere to substitution therapy and/or utilize substance, which impact improvement in outcomes. Traditional toxicology testing in these specialties includes appropriate prescription medications as well as illicit substances such as heroin, cocaine, methamphetamine, LSD, and PCP. The intent of this testing in these environments is to offer a healthcare practitioner the opportunity to address diversion, substance misuse, or non-adherence to therapy before situations escalate. Toxicology laboratories providing services to physician offices have traditionally offered NPS testing to include synthetic cannabinoids and cathinones, with some offering fentanyl analog tests recently. However, over the last several years, the NPS marketplace has grown to include numerous substances, such as non-fentanyl-based opioids, benzodiazepines, hallucinogens, and dissociatives, to name a few. ***Methods***: This study is a retrospective data review of samples tested for novel psychoactive substances from January 2021 through July 2021. NPS of interest, to include designer benzodiazepines, opioids and xylazine were analyzed in the laboratory as part of a larger NPS panel. Target analytes were included in the method based on information from the US Drug Enforcement Administration and other online resources. Prior to analysis, analytes were extracted from hydrolyzed urine using a liquid-liquid extraction followed by evaporation and reconstitution in mobile phase. Samples were injected onto a liquid chromatograph/tandem mass spectrometer (LC-MS/MS) instrument consisting of a Shimadzu Prominence HPLC and Sciex API 4000 MS/MS. The mass spectrometer was operated in positive electrospray ionization mode for scheduled multireaction monitoring (sMRM) analysis. Analytes were chromatographically separated on a Biphenyl column. Upon request by a provider during the study period, samples were analyzed for designer benzodiazepines, opioids and xylazine, with LODs ranging from 1-25 ng/mL. ***Results/Conclusion*****:** Final analysis yet to be completed, but data/presentation will include**:**

- The detection of selected NPS of interest, to include opioids, benzodiazepines and xylazine, along with co-positivity for traditional toxicology testing, such as buprenorphine.
- The process required to keep test menus as current as possible, while maintaining efficiency in a high-throughput laboratory.
- Select instances when detection in a healthcare laboratory mirrors external sources of prevalence/drug detection.