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In recent years, there has been a dramatic increase in the number of new psychoactive substances (NPS) detected across the world. The NPS market remains highly dynamic and is characterised by the emergence of large numbers of new substances reported in a growing number of countries. Between 2009 and 2018, 119 countries and territories reported the emergence of 950 NPS to the United Nations Office on Drugs and Crime (UNODC), through the UNODC Early Warning Advisory on NPS. In Europe, since 1997, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), has been monitoring and responding to NPS appearing in the EU through the EU Early Warning System on NPS. At the end of 2019, the EMCDDA was monitoring around 790 NPS, 90% of which were identified for the first time in the last decade. The increase in the number and availability of NPS in recent years has largely been driven by globalisation, the internet and rapid changes in technology. Yet, recently, there have been some encouraging signs. For example, the number of NPS reported annually for the first time in Europe has dropped from a high of around 100 in 2014 and 2015 to around 50 in 2017, 2018 and 2019. Nevertheless, NPS continue to pose numerous challenges in terms of detecting and monitoring; understanding patterns of use and harms caused; and developing appropriate public health responses. In Europe, around one new substance is still detected every week, increasing the overall number that needs to be monitored. Major new problems have also emerged, that have led to an increasing number and range of risks for people who use psychoactive substances. These include an increase in the number of highly potent NPS on the market — many of which are synthetic cannabinoids or synthetic opioids — and the COVID-19 pandemic. Given the numerous challenges posed by NPS, we are pleased to launch the International Society for the Study of Emerging Drugs (ISSED) to strengthen multidisciplinary and international collaboration to enhance knowledge and improve the quality of information-sharing in this complex area in a more coordinated way. In this context, the NPS conference series, now in its 7th edition, is the major international forum on NPS, attracting hundreds of participants worldwide. This year’s conference is jointly organised by the ISSED, UNODC, the EMCDDA, the World Anti-Doping Agency (WADA), the University of Hertfordshire (UH) and the Centre for Forensic Science Research & Education (CFSRE).
CONFERENCE ABSTRACTS

(in alphabetical order)

Image Enhancing Drugs: an overlooked phenomenon?
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Background: In a society that strives for beauty and perfection, people are increasingly adopting risky behaviours to enhance their body image. One of these behaviours is the use of skin-lightening products. Those products are available to buy from unregulated internet websites with no medical prescriptions. Some of these products might contain contaminants, or undisclosed ingredients, which are responsible for skin or systemic side-effects. On the other hand, little is known about the motivational factors behind this risky practice including the presence of psychological problems such as Body Dysmorphic Disorder (BDD) among others. Methods: Mixed qualitative and quantitative approaches have been used in clinical and non-clinical settings. After an initial literature review, an internet-based research has been carried out in beauty and skin care websites. Also, a semi-structured questionnaire has been administered online and in dermatology clinics. In addition, participants who are interested have been invited to attend a face-to-face in-depth semi-structured interview with the researcher. Results: Results of the thematic analysis of both online forums data and face to face interviews have yielded three prominent motivational themes behind the practice of skin lightening which are 1) Sociocultural related factors including getting influenced from other people or online resources, 2) Skin related conditions especially hyperpigmented lesions, and 3) Psychological related factors including body image concerns. Also, it has been revealed that skin lightening agents are divided into topical which are easily found agents online or in stores and systemic ones e.g. intravenous treatments which are available from online resources and some clinics. Conclusions: Many skin side-effects have been reported with skin lightening products use such as thinning of skin, visible veins, stretch marks, irritation, peeling, patchy discoloration, acne, pimples, etc. Moreover, skin lightening can be accompanied by bullying experience and other psychological impacts. Results of the quantitative data are in progress and will be revealed at the day of conference.

International Snapshot of Novel Psychoactive Substance Use: Case study of ten countries over the 2019/2020 New Year Period
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Background: Despite the best efforts of government legislation, novel psychoactive substances (NPS) are continually evolving, with almost 1000 NPS having been reported to the UNODC. With little to no information available on “safe” doses, side effects and toxicity, it is important to monitor community consumption of these substances, particularly over festive periods. Wastewater analysis has proven itself invaluable in this regard, due to its ability to anonymously monitor consumption within a community through the wastewater. An international study was thus carried out by sampling simultaneously in ten countries over the 2019/20 New Year period to investigate which NPS are most prevalent and whether any geographic patterns are evident. Methods: Influent wastewater samples were collected from up to four sites per country in ten countries (Australia, United States, New Zealand, Italy, Spain, Norway, China, Belgium, the Netherlands and the United Kingdom) from December 26, 2019 – January 3, 2020. Samples were loaded onto solid phase extraction cartridges in the countries of origin before being shipped to the University of South Australia for elution and sample analysis. All samples were analysed with two liquid chromatography - mass spectrometry methods. One employed a (semi)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 nine NPS were semi-quantified in the samples: 4-fluoroamphetamine, 4-methylethcathinone, methedrone, 3-methylmethcathinone, N-ethylpentylone, methcathinone, ethylone, pentylone and methylone. A further seven were qualitatively found: 4-fluoromethamphetamine, 4-chloromethcathinone, mitragynine, ketamine, norketamine, acetyl fentanyl and etylcynone. In samples from Norway and China, only ketamine and its metabolite norketamine were seen. N-ethylpentylone was the most prevalent NPS being found in New Zealand, Australia and the United States. Methedrone had the highest mass load of all NPS. It is interesting to note the geographic specificities of some of the substances – 3-methylmethcathinone was only seen in Spain, the Netherlands and Italy; methedrone and etylcynone in New Zealand, United States, the Netherlands and Australia; 4-chloromethcathinone in Italy, 4-fluoroamphetamine, 4-methylethcathinone and 4-fluoromethamphetamine in the Netherlands and mitragynine and acetyl fentanyl in the United States. Conclusions: This work has shown the utility of wastewater analysis to monitor community consumption of NPS. A snapshot into the consumption of NPS consumption in ten countries was made by analysing samples over the 2019-20 New Year period. A total of 16 substances were found, with synthetic cathinones the most common.
Analytical, pharmacological, and toxicological evaluation of prevalent synthetic cannabinoid receptor agonists
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Background: More than 250 synthetic cannabinoid receptor agonists (SCRAs) have been identified in recreational drug markets across the globe since the first examples were detected a decade ago. SCRA s are increasingly associated with mass intoxications involving severe illness and death. Methods: We have used a modular, divergent synthetic approach to rapidly and proactively develop a library of recent SCRA s and metabolites based on structural trends in the SCRA marketplace. The SCRA library was screened at human cannabinoid type 1 and type 2 receptors (CB1 and CB2, respectively) using radioligand binding assays and fluorescence-based plate reader membrane potential assays, and evaluated in mice using radiobiotlemetry. Through the Psychoactive Surveillance Consortium and Analysis Network (PSCAN), we have used liquid chromatography–quadrupole time-of-flight–mass spectrometry (LC-QTOF-MS to screen for all members of the library in patient samples from multiple partner sites in the US. Results: Many of the most recently detected SCRA s, such as CUMYL-CBMICA, ADB-BUTINACA, 4F-MDMB-BINACA, AMB-4en-PINACA, and 5F-AB-P7AI C A, showed nanomolar affinity for CB1 and CB2 receptors. Unlike Δ9-THC, most recent SCRA s were found to act as high efficacy agonists of CB1 and CB2, with greater potency than Δ9-THC itself. Moreover, many of these compounds demonstrated potent cannabimimetic activity in mice, inducing central CB1-mediated hypothermia, hypolocomotion, and bradycardia with greater potency than Δ9-THC. Several SCRA s showed potent proconvulsant activity in mice, consistent with reports of seizure in human users of these substances. PSCAN has detected many SCRA s from this library in patient blood samples, including derivatives not yet reported by forensic chemistry groups, underscoring the value of a proactive approach to the synthesis and characterization of emerging SCRA s and their analogues, and the clinical confirmation of SCRA s in toxicology cases. Conclusions: Structure-activity relationships for the CB1 and CB2 receptor binding and agonist activity of the most recent and prevalent SCRA s, including CUMYL-CBMICA, ADB-BUTINACA, 4F-MDMB-BINACA, AMB-4en-PINACA, 5F-AB-P7AI C A and their analogues have been identified. Key structural features contributing to CB1 agonist potency in vitro and in vivo were determined. Several SCRA s identified in illicit drug markets were observed to cause seizures in mice, consistent with clinically noted adverse effects in humans. The proactive generation of SCRA analogue libraries, and the utility of such libraries to the rapid detection of emerging drugs of abuse by clinical toxicologists, was demonstrated. The unprecedented potency and efficacy of many recently identified SCRA s at CB1 receptors may contribute to the clinical toxicity of these substances.

Designer Benzodiazepines: What's In Fake "Xanax"?
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Not available

Identification of isotonitazene, the first member of a novel emerging class of legal, highly potent benzimidazole NPS opioids
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Background: We report on the identification and full chemical characterization of isotonitazene (N,N-diethyl-2-[5-nitro-2-[(4-[(propan-2-yl)oxy]phenyl]methyl]-1H-benimidazol-1-yl]ethan-1-amine), the first member of a new class of (ultra)potent benzimidazole opioids, legally available online on markets. Methods and Results: Identification of isotonitazene was performed by gas chromatography mass spectrometry (GC–MS) and liquid chromatography time-of-flight mass spectrometry (LC-QTOF-MS), the latter identifying an exact-mass m/z value of 411.2398. All chromatographic data indicated the presence of a single, highly pure compound. Confirmation of the specific benzimidazole regio-isomer was performed using 1H and 13C NMR spectroscopy, after which the chemical characterization was finalized by recording Fourier-transform (FT-IR) spectra. A live cell-based reporter assay to assess the in vitro bio-logical activity at the μ-opioid receptor (MOR) revealed that isotonitazene has a high potency (EC50 of 11.1 nM) and efficacy (Emax 180% of that of hydromorphone), thus confirming that this substance is a very potent opioid. Conclusions: Isotonitazene has not been previously detected, either in powder form, or in biological fluids. The high potency and efficacy of isotonitazene, combined with the fact that this compound was being sold undiluted, represents an imminent danger to anyone ordering legal opioids online.

Ambient ionization mass spectrometry applied to new psychoactive substance analysis
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Background: In the past decade, the world has seen the emergence of new drugs that have similar effects to controlled psychoactive drugs, like amphetamines or lysergic acid diethylamide. These drugs can collectively be classified under
the term new psychoactive substances (NPS) and are used for recreational purposes. The novelty of the substances, alongside the rate of emergence and structural variability, makes their detection as well as their legal control more difficult, increasing the demand for rapid and easy-to-use analytical techniques for their detection and identification. Therefore, interest in ambient ionization mass spectrometry (AIMS) applied to NPS has grown in recent years. This is largely because of the relatively fast, simple and low operating cost of AIMS, allowing the ionization of the analyte(s) of interest at atmospheric pressure from unprepared samples. This review aims to provide an updated overview of the current ambient ionization techniques’ suitability for the analysis of NPS in the forensic and clinical toxicology field. Methods: In order to do so, peer-reviewed primary studies of the last 10 years in various databases were examined and a thorough appraisal of the findings was carried out. Results: The applicability of AIMS techniques to NPS analysis was found to be mainly compromised by ionization inefficiency, strong ion suppression, poor selectivity, low reproducibility and the inability to distinguish isomeric and isobaric molecules. Conclusions: With further research directed at improving the instrument design and at understanding the effect of sample complexity on the analyte of interest, AIMS show potential as an alternative technique providing minimal to no sample preparation and fast analysis time in screening applications.

**Emerging drugs harms: new app and tools to train clinicians**
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Not available

**Semi-quantitative activity-based detection of JWH-018, a synthetic cannabinoid receptor agonist, in oral fluid after vaping**
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Background: The rapid proliferation of new synthetic cannabinoid receptor agonists (SCRAs) has initiated considerable interest in the development of so-called “untargeted” screening strategies. One of these new screening technologies involves the activity-based detection of SCRAs. In this study, we evaluated whether (synthetic) cannabinoid activity can be detected in oral fluid (OF) and, if so, whether it correlates with SCRA concentrations. Methods: OF was collected at several time points in a placebo-controlled JWH-018 administration study. The outcome of the cell-based cannabinoid reporter system, which monitored the cannabinoid receptor activation, was compared to the quantitative data for JWH-018, obtained via a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Results: A total of 175 OF samples were collected and analysed via both methods. The cannabinoid reporter assay correctly classified the vast majority of the samples as either negative (<0.25 ng/mL; 74/75 = 99%), or having low ((0.25 – 1.5 ng/mL; 16/16 = 100%) and 1.5 – 10 ng/mL; 37/41 = 90%), mid (10 – 100 ng/mL; 23/25 = 92%) or high (>100 ng/mL; 16/14 = 89%) JWH-018 concentrations. Passing-Bablok regression analysis yielded a good linear correlation, with no proportional difference between both methods (slope 0.97; 95% confidence interval 0.86 – 1.14) and only a small systematic difference. Conclusions: This is the first study to demonstrate the applicability of an untargeted, activity-based approach for SCRA detection in OF (Cannaert et al. 2020 Anal Chem). Additionally, the outcome of the cannabinoid reporter assay was compared to the gold standard (LC-MS/MS), showing a good correlation between both methods, indicating that the cannabinoid reporter assay can be used for an estimation of drug concentrations.

**NPSfinder® designer benzodiazepines: QSAR and Docking studies**
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Background: The non-medical use of benzodiazepines (BZDs) is considered a threat to public health. From 2017 to 2020, a rise in the use of designer/NPS benzodiazepines (DBZDs) was reported and evidence of health and social harms associated with their use/abuse published. DBZDs are strong CNS depressants and, when used in combination with other drugs, cause serious toxicity with profound sedation, respiratory depression, coma, and death. At date, the officially identified and reported (UNODC, EMCDDAA) DBZDs are 31. However, a web crawling exercise over the surface web identified a much higher number of DBZDs, precisely 130. This finding amplifies the concern towards DBZDs with regards to their unknown potency, pharmacological and toxicological effects. The aim of this study was to generate QSAR models and conduct a molecular docking study, to investigate/predict the biological activity of these 131 DBZDs on the GABA-A benzodiazepine receptor. Methods: The web crawling activity was conducted with the use of an ad hoc software, NPSfinder®, created by the Italian IT company Damicon. QSAR and Docking studies were performed with MOE. To build robust training and test sets for QSAR, 78 BDZs were divided with the use of Tanimoto coefficients. Their experimentally derived biological activity values (log 1/C) were used to build QSAR models. 3D crystallised GABA-A receptor-ligand complexes from the Protein Data Bank (PDB) were used for the docking. For each of the 130 DBZDs, an “S” value was generated that served as an indication of binding affinity. Results: The best QSAR model was
used to predict the log 1/C values for the 130 DBZDs. Of these, 74 were found to be higher than Alprazolam (high potency BZD, log 1/c=7.7) suggesting a strong activity on the GABA-A. These findings were compared to the docking: some 60 DBZDs showed a stronger binding affinity than Alprazolam. These 60 DBZDs (e.g. Cinazepam, Mpi-iii-022, Ethyl carfuzapate, Renimazolam, etc) share some common features that can be hypothesised as important for a good binding affinity: an halogen or NO₂ substituent on the C7 carbon of the benzodiazepine backbone, a long/bulky substituent on the C3, a double bond (C=O) or a five member ring substituent on C2. Conclusion: QSAR and docking studies recognised a large number of possibly very potent DBZDs among the ones identified on the surface web. Further studies are necessary to investigate/understand the relation between chemical features (pharmacophore) and biological activity and evaluate the threat that DBZDs may pose.

Beyond the purple drank. Study of promethazine abuse according to the euadravigilance dataset
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Background: Promethazine is a medicinal product, available on its own or in combination with other ingredients including dextromethorphan, paracetamol, and/or expectorants. Anecdotal reports have however indicated that promethazine may have a misuse potential, especially in adolescents. Objective: We aimed at studying how this phenomenon has been reported to the European Monitoring Agency (EMA) Adverse Drug Reactions (ADRs) database. Methods: After a formal request to the EMA, the promethazine-specific dataset has been studied performing a descriptive analysis of the misuse/abuse/dependence related-ADR reports. The study was approved by the University of Hertfordshire (LMS/PGR/UH/03234). Results: The analysis of promethazine data showed increasing levels of misuse/abuse/dependence issues over time (2003–2019). Out of a total number of 1,543 ADRs’ cases, the abuse/misuse/dependence-related cases reported were 557, with ‘drug abuse’ (300/557: 53.8%) and ‘intentional product misuse’ (117/557: 21.0%), being the most represented ADRs. A high number of fatalities were described (310/557: 55.6%), mostly recorded as ‘drug toxicity/drug abuse’ cases, with opiates/opioids having been the most commonly reported concomitant drugs used. Conclusions: Anecdotal promethazine misuse/abuse reports have been confirmed by EMA data. Promethazine misuse/abuse appears to be an alarming issue, being associated with drug-related fatalities. Thus, healthcare professionals should be warned about a possible misuse of promethazine and be vigilant, as in some countries medicinal products containing promethazine can be purchased over the counter. Since promethazine is often available in association with opioids, its abuse may be considered a public health issue, with huge implications for clinical practice.

NPS in Europe
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In Europe, a three-step legal framework of early warning, risk assessment, and control measures allows the European Union (EU) to rapidly detect, assess, and respond to the public health and social threats caused by new psychoactive substances. The EMCDDA is responsible for the first two steps in this system, namely, operating the EU Early Warning System (EWS) on New Psychoactive Substances (NPS) in close cooperation with Europol, and conducting risk assessments. The European Commission is responsible for proposing control measures. The EU EWS is composed of a multi-agency and multidisciplinary network, which includes the EMCDDA, 30 national early warning systems, Europol and its law enforcement networks, the European Medicines Agency (EMA), the European Commission, and other partners. Underpinning each of the national early warning systems (NEWS), and, in turn, the EU EWS, is the exchange of information on the chemical identification of new psychoactive substances from forensic and toxicology laboratories. This approach allows the collection and rapid reporting of event-based information on the appearance of, and, harms caused by, NPS at national level to the EMCDDA. These data are complemented by biannual reports, which include aggregated data on seizures by law enforcement and from poisonings. There have been some encouraging developments in the NPS market in Europe that have been particularly visible from around 2015 onwards. These include a decrease in the number of new substances identified for the first time each year and an overall decrease in seizures, especially driven by a drop in seizures of synthetic cannabinoids and cathinones — the two largest groups of new substances monitored by the EMCDDA. In part, these changes appear to be related to a disruption in the ‘legal high’ trade, which for a period saw NPS sold openly on the high street in many countries in Europe. More generally, broader policy responses designed to restrict the availability of new psychoactive substances are also likely to have had an effect. However, since 2015 a greater proportion of substances associated with problematic use — particularly opioids and benzodiazepines — have appeared on the market bringing a new set of problems; while the market in synthetic cannabinoids, once the epitome of the ‘legal highs’ phenomenon, has also evolved to pose a threat to health security. During this time, the NPS market has also developed stronger links with the markets in established controlled drugs.

Unsupervised supplement intake: an underestimated phenomenon in online fitness settings
Background: Supplements use is widely spread among online fitness settings. Particularly, a wide range of vitamins, minerals, amino acids or other “ergogenic aids”, which can have both short-term and long-term side effects, are promoted and sold with misleading information on the support of weight-loss, muscle gain and performance enhancement. Our study focused on the popularity of such compounds in online communities, investigating the main sources of information, health risks awareness and possible side effects, through the thematic analysis of internet fora. Methods: A thematic qualitative analysis was conducted on 4 popular fitness fora, which were chosen according to (i) relevance of the research objective, (ii) activity of the forum and (iii) number of posts (at least 7 per week). Five of the most popular sport supplements (creatine, multivitamins, whey protein, nitric oxide, branched-chain amino acids) were used as keywords for the analysis. Thematic analysis was performed following five themes: motivations, availability of information, side effects, perceived risks of health, contamination issues. Results: The analysis of fitness fora revealed discussions on a considerable number of perceived side effects, health and psychological risks associated with supplement intake, such as acne and water retention, weight gain, kidney damage, addiction, etc. Among the sources of information, 21% of fora users referred to obtain information from websites and social media, while the 17% relied on fitness influencers’advices. Main motivations of intake were muscle gain (23%) and increased energy (10%). Conclusions: Websites and social media are among the main sources of supplements’ promotion, with “fitspiration” trends in social media playing a relevant role. Additional investigations on supplements intake on online fitness settings are required to further elucidate the extent of harm, especially for young people, as well as to support the development of more targeted preventive strategies.

Use of novel psychoactive substances (NPS) of natural origin: an international survey
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Background: NPS recreational uses are mostly derived and modified from constituents of natural origin. Here we investigated the motivation of natural NPS use, perception of potential associated health risks, and demographic factors associated with natural NPS use. 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 1 July and 17 November 2018 (812 responses). This pharmacoepidemiologic study was evaluated using the SPSS software (IBM SPSS Statistics version 24; MacOS Sierra 10.12.3). Results: The main motivation (67%) for natural NPS use was curiosity to “experience something new and different” with a low perception of health risk (83%). The preferred natural NPS was magic mushrooms (psilocybin, 95%) often in combination with cannabis (63%). Gender, living area, educational background, smoking frequency and employment significantly affected (P<0.001) natural NPS use. Male respondents, residents of suburban and rural areas, smokers and respondents with low educational level represented the majority of natural NPS users as well as the employed, the unable to work and retired groups. Similarly, sexual orientation significantly affected (p<0.05) natural NPS use. Conclusions: Users’ low perception of natural NPS safety profile and the fact that natural NPS use correlates with a lower level of education, indicates a need for enhanced statutory targeted prevention interventions in schools. Many users (67%) reported natural NPS make them be happier and more optimistic about life emphasising the need to study the potential application of these substances in appropriate clinical settings for therapeutic purposes in mental health.

Herbal based psychoactive substances in Nigeria: a case for more research in Africa
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Background: Studies indicate that although the use of novel psychoactive substances (NPS) is on the increase in Africa, there is little data or research in the area. Also, contemporary studies on NPS often preclude studies in Africa. This may not be unrelated to the ‘non-classical packaging’ of NPS in homemade and herbal mixtures. The morbidity and mortality associated with such psychoactive substances have been subject of many clinical reports and studies. This study aims to explore and highlight the non-classical presentation of NPS in Nigeria and the implications for young people in Nigeria. Methods: The data were collected from two exploratory studies. First, an online interview method was used to obtain information from 10 young adults (20-35 years) in Southern Nigeria. Additionally, 23 young adults (23-29 years) were interviewed on a face-to-face basis in Eastern Nigeria. The data were analysed thematically. Results: The participants gave detailed lists of NPS used among young people in contemporary Nigeria. Consuming drug cocktails and ‘concoctions’ was common among the participants and their friends. Some used what they called Codeine Diet (i.e., Codeine-based cough syrup mixed with Malt or Coca-Cola drinks), while others took Gutter Water (a cocktail of cannabis, Codeine, Tramadol, vodka, and juice or water). The use of Monkey Tail (a mixture of local gin, cannabis leaves,
stems, roots, and seeds), petrol mixed with glue and La Casera (carbonated soft drink) combined with Tom-Tom (a popular menthol-flavoured candy) was also revealed. Pleasure, better highs, perceived prolonged intoxication, and the use of one drug to dose the effects of another substance motivated the use of some of these substances. Conclusions: In resource-poor settings, it may be easy to ignore NPS if emphasis is placed on its classical definition. Few studies have focused on the chemical assay of these drug cocktails in our setting. Although a few case reports have highlighted the clinical sequelae of ingestion of these drug cocktails, there is a dearth of research on the short- and long-term consequences in Nigeria. It is pivotal that governments, research, and funding organisations commit resources to an in-depth systematic exploration of the impact of homemade psychoactive substances in low-resource settings.

**How to detect the use of a new illicit substance by combining different strategy**

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Detecting the appearance or use of a new psychoactive substance is not always easy. There are several sources of data which are of various nature either linked to a physical environment (analysis of police seizures, analysis of waste water, analysis of used syringes...) or to a digital environment (analysis of the darknet, analysis of the forum...). There are also many actors (law enforcement, border units, medical emergencies) who have essential data to identify a new phenomenon. In this presentation, we will discuss the deployment of different strategies as well as a proposal for the articulation of these different sources of information in order to detect NPS or follow the evolution of a specific problem.

**Adulteration of Drugs with Toxic Cutting Agents: An Emerging Global Public Health Threat**

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The last decade has witnessed an outbreak of public health problems resulting from toxic adulterants being added to illicit drugs. Some of these adulterants (phenacetin, aminopyrine) are pharmaceuticals that were added to aspirin, but banned in the 1960s due to problems such as kidney failure, bladder cancer, and reduction of both red and white blood cells. Another adulterant, levamisole, is a de-worming agent for cattle that depletes white blood cells in humans leading to depressed immune systems and various infections. These new adulterants include, but are not limited to: banned pharmaceuticals, veterinary products, analgesic pain relievers, sedatives, antihistamines, opioid pain medications, muscle relaxants, antiarrhythmics, and impurities from the heroin manufacturing process. These compounds have been associated with severe health effects, including: decreased production of red and white blood cells due to bone marrow damage, multifocal inflammatory leukoencephalopathy, hemolytic uremic syndrome, renal failure, multiple malignancies, life-threatening cardiac arrhythmias, overdose, and death. This presentation will address the appearance of these toxic adulterants in different regions of the world and the unique threats they pose to public health.

**Functional and histological changes in rat urinary bladder following short- and long-term Ketamine exposure**

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Background: Due partly to their dissociative properties, ketamine and related new psychoactive substances (NPS) are widely used as recreational drugs. This has led to the discovery of a link between the chronic use of ketamine and methoxetamine and the development of cystitis, which is typically associated with an array of lower urinary tract symptoms indicative of an overactive bladder. Interestingly, however, in vitro organ bath experiments demonstrate that acute exposure to ketamine causes relaxation in human bladder. Ketamine has been shown to dose dependently inhibit Ca2+ influx through L-type calcium channels in various tissues and species, and so this may contribute to the relaxant effects observed following acute exposure. Here, the effects of short term (20 minutes) and longer term (72 hours) ketamine exposure on carbachol-induced rat urinary bladder contractility are assessed in an in vitro organ bath assay. The mechanisms behind the functional changes observed were also investigated by looking at the effects of ketamine following blockade of L-type calcium channels; and through immunohistological assessment of L-type calcium channels. Methods: Rat bladder was dissected into longitudinal strips and suspended in organ baths containing 15mL Krebs-Hensesli solution, supplied with 95% O2 5% CO2, and maintained at 37°C. Strips were contracted with increasing concentrations of carbachol and then incubated with ketamine (up to 3mM) for 20 minutes within the organ bath, or 72 hours in an incubator, before carrying out another carbachol dose response. For histological assessment, rat bladder was dissected in half longitudinally and cultured at 37°C with 3mM ketamine for 72 hours, with the other half of the bladder acting as a time matched control. Bladders were then fixed in 10% formalin for 24 hours, dehydrated through graded alcohols and cleared with xylene, embedded in paraffin wax, sectioned at 5µM thickness and stained for immunohistochemical visualisation of L-type calcium channels. Results: Acute exposure to ketamine (≥300µM up to 3mM) decreased the contractile response of bladder strips to carbachol — a response that is predominantly L-type calcium channel dependent. In contrast, longer term 3mM ketamine exposure significantly increased contractility of bladder,
compared to control tissues. The longer-term effects of organ culture and ketamine on L-type calcium channels will be presented. Conclusions: This is the first study to demonstrate in vitro rat bladder overactivity following ketamine exposure. Further results will provide insight into the mechanisms behind the functional and histological bladder changes induced by ketamine.

NPS in the time of COVID-19
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Not available

Laboratory diagnostics of acute poisoning with new synthetic cathinones (MDPHP, α-PBP) in the Russian Federation
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Background: In 2018-2020, cases of acute poisoning were recorded in the Russian Federation associated with the use of MDPHP(3,4-methylenedioxy-α-pyrrolidinovalerophenone,1-(benzo[d][1,3]dioxol-5-yl)-2-(pyrrolidin-1-yl)hexan-1-one) and α-PBP(α-pyrrolidonobutilphenone; 1-phenyl-2-(pyrrolidin-1-yl)butan-1-one), which are homologues of the narcotic drugs of 3,4-methylenedioxyphynorvalerone (MDPV) and α-pyrrolidinovalerophenone (α-PVP), respectively. The aim of the investigation was to study the metabolic pathways of MDPHP and α-PBP in the laboratory diagnosis of acute poisoning. Methods: Seven urine samples were collected from patients with acute poisoning with synthetic cathinones in different regions of Russia. MDPHP and α-PBP and its metabolites were detected and identified by gas chromatography-mass spectrometry and high-resolution liquid chromatography - tandem mass spectrometry (LC - HRMS/MS). Results: The major pathways of biotransformation for α-PBP and MDPHP were similar to those other synthetic cathinones. It was found that α-PBP and MDPHP undergo extensive metabolism with the formation of a large number of products of the 1st and 2nd phase of biotransformation. Metabolites resulting from demethylation and subsequent methylation (MDPHP), reduction of carbonyl group (α-PBP) and oxidation to form a lactam combined with ring-opening (α-PBP and MDPHP) were found to be the most useful target analytes for the confirmation of ingestion. The majority of the hydroxylated metabolites of α-PBP and MDPHP were found to be glucuronidated. Conclusions: The main pathways of metabolism of 3.4-MDPHP and α-PBP were studied. The obtained data can be used to detect and identify new synthetic cathinones in the biomaterial of patients with acute poisoning.

Identification, detection and monitoring: What’s next?
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The UK’s First Home Office-Licensed and Pharmacist-Led Community Drug Checking Service
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Background: Drug-related deaths related to illicit use are soaring with no sign of abating. Emerging psychoactive substances with unpredictable adverse effects are still emerging and are posing serious public health risks. On-site “drug checking” services in the UK have predominantly operated at festivals without a licence by the Home Office. AIMS: The aim of this study was to pilot the UK’s first pharmacist-led, Home Office-licensed community drug checking service to enable pharmaceutical analysis and tailored advice in real time. Methods: A bespoke protocol incorporating legally, professionally and ethically binding documents was implemented. A free, anonymous, confidential service was designed for anyone over 18 who provided informed consent and who agreed to supply a non-returnable drug sample. Samples were checked on-site at an established substance misuse service (SMS) using a portable laboratory device to determine the likely drug and adulterant content. In parallel, participants completed a questionnaire about their substance use and the drug sample(s) being tested. A pharmacist-led multidisciplinary approach was adopted to discuss the pharmaceutical analysis findings and communication of the results to the participant, based on which a tailored harm reduction intervention was delivered. Results: The pilot operated for four days over four weeks. Eleven people visited and relinquished a total of thirteen samples. Half of the participants had previously overdosed and were known to the SMS. Seventy percent were male, all were White British 30% were employed and two people disclosed visiting from another nearby town. Samples included what was thought to be heroin, synthetic cannabinoids, stimulants, benzodiazepines and LSD and none required activation of the ‘alerts cascade’. Most people drank alcohol regularly with concomitant use of traditional illicit drugs and prescribed medication (including opioids, anxiolytics and antidepressants) with sedating profiles was common. Subjects of different ages and patterns of drug use accessed the service. Given some of the ethical decisions and interpretation of the results, specialist clinician involvement was deemed essential. Conclusions: This pilot
Background: Over the past few years, there has been an increase in detection of various novel psychoactive substances (NPS) in toxicology cases. To avoid regulations, new substances are introduced to the street daily by simple structural modification of known drugs. The Orange County Crime Lab (OCCL) in California is recording a higher count of NPS each year with an emphasis on benzodiazepines and mitragynine. Methods: Blood and urine samples submitted to the laboratory received screening by Immunalysis Direct enzyme-linked immunosorbent assay kits for amphetamine, methamphetamine, THC, and barbiturates. Starting in August of 2018, additional screening by liquid chromatography quadrupole time of flight (LC-QTOF) was also performed to analyse for prescription, illicit and over-the-counter drugs. Included in this specific broader screen are designer benzodiazepines, novel opioids, bath salts and synthetic hallucinogens. Since the LC-QTOF screen has been brought on-line, new synthetic compounds were added when they were identified by the Seized Drug section to expand the screen. Positive screened samples received confirmation by either gas chromatography mass spectrometry (GCMS) or liquid chromatography tandem mass spectrometry (LCMSMS) instrumentation. Results: From 2017 to 2019, 33,753 ante-mortem and post-mortem cases were received and analysed for designer drugs. An increase of appearances of various designer benzodiazepines, clonazolam, etizolam, and flubromazolam are seen, with flubromazolam having 17 positive samples in 2018 to 57 samples in 2019. Analogues of fentanyl, such as acetylfentanyl, β-hydroxy fentanyl and valeryl fentanyl, also increased; with two cases of valeryl fentanyl in 2018 and 11 cases in 2019. Mitragynine prevalence also increased more than 80% from 2018 to 2019. Designer benzodiazepines are seen in both DUID and post-mortem cases; fentanyl analogues are more prevalence in DUID cases, while mitragynine is more common in post-mortem cases. Conclusions: Detecting NPS is important to toxicology laboratories to get a true view of what drugs are being abused. The addition of exact mass instrumentation and the collaboration with a Seized Drug section aid in determining in what is currently being used in the community. Within three years, OCCL went from detecting very few of these drugs to increasing more than 80% for most of the NPS they detect. This increase not only had to do with utilizing new instrumentation, but also working more closely with the Seized Drug Section. The most common NPS class seen in Orange County, California are designer benzodiazepines, with flualprazolam being the most common in the first half of 2020.

### Novel designer drug 25I-NBOMe affects neurotransmission and induces neurotoxicity in the rat frontal cortex

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Background: Hallucinogens, a class of novel psychoactive substances, potently alter mood and perception but do not produce addiction and dependence. NBOMes are N-benzylmethoxy derivatives of the 2C family hallucinogens that mimic LSD effect. 25I-NBOMe (4-iodo-2,5-dimethoxy-N-(2- methoxyphenyl)phenethylamine) exhibits high binding affinity for 5-HT2A/2C serotonin receptors. Many case studies confirm serious toxicity and fatalities after 25I-NBOMe intake in humans. However, the knowledge of NBOMes effect on the central nervous system is very limited. Therefore, the aim of our study was to investigate the impact of 25I-NBOMe on brain neurotransmission and possible induction of neurotoxicity. Methods: We examined the effect of 25I-NBOMe single doses on extracellular levels of dopamine (DA), serotonin (5-HT) and glutamate in the rat frontal cortex using microdialysis in freely moving animals. Neurotoxic properties of 25I-NBOMe were determined by measurement of tissue content of DA and 5-HT, their metabolites (DOPAC, HVA and 5-HIAA, respectively), and nuclear DNA damage assessed with the Comet Assay. Furthermore, 25I-NBOMe presence was analyzed with electrospray ion trap mass spectrometry in the rat plasma and frontal cortex.

Statistical analysis was done using Student's t-test or repeated measures ANOVA and comparisons between groups were calculated with Tukey post hoc test. Results: Acute administration of 25I-NBOMe increased release of DA, 5-HT and glutamate in non-linear manner. It increased 5-HT and 5-HIAA cortical content but did not affect DA, DOPAC and HVA tissue levels. The oxidative damage was observed by DNA double and single-strand breaks occurrence. 25I-NBOMe was detectable in the blood plasma and rat frontal cortex 15 min after injection. The concentration of the compound was higher in the plasma than in the frontal cortex and was dose-dependent. The observed inverted U-shaped dose-response effect on DA and 5-HT and U-shaped effect on glutamate levels seem to result from activation, apart from 5-HT2A, 5-HT2C receptors by 25I-NBOMe. It is suggested that higher doses of 25I-NBOMe activate 5-HT2C receptor subtype causing release of GABA. The inhibitory impact of GABA on pyramidal cells modulates glutamate release that indirectly causes changes in DA and 5-HT levels. Observed DNA double and single-strand breaks indicate generation of oxidative stress by 25I-NBOMe. However, the lack of tissue monoamine deficits does not point to long-lasting neuronal damage. Conclusions: Mass spectrometry data show that 25INBOMe easily crosses the blood-brain barrier which results in its immediate effect on neurotransmission.
Background: New psychoactive substances (NPS) among the synthetic opioids, synthetic cannabinoids, synthetic cathinones, designer benzodiazepines, and other drug classes continue to pose threats to the public health and safety in the United States and countries around the world. Over 950 NPS were identified in the past decade, which are being monitored domestically in the United States. Methods: We will present the current NPS trends of several major drug classes in the United States along with forecasts based on data 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 U.S. Finally, we will highlight the timelines of some select substances that have emerged in the U.S. illicit drug market. Results: The impact on the rate of new NPS emerging in the U.S. illicit drug market is evaluated following multiple temporary control actions on NPS and a temporary control on fentanyl-related substances (FRS) as a class. The number of FRS reports to NFLIS-Drug have noticeably decreased. Reports of new synthetic cannabinoids and benzodiazepines have increased in recent years. Select substances highlighted will include some FRS, isometitizine, MDMB-4en-PINACA, and flualprazolam. Conclusions: Early identification of NPS emerging on the illicit market requires close collaboration, efficient communication, and the new modality of early warning systems to mine, analyse and model data for signals and trends. Challenges remain in this rapidly changing landscape, but there have been successes and lessons learned.

In vitro activity-profiling of Cumyl-PEGACLONE variants at the CB1 receptor: fluorination versus isomer exploration
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Background: Two relatively recent synthetic cannabinoid receptor agonists (SCRAs), Cumyl-PEGACLONE and 5F-Cumyl-PEGACLONE, were not controlled by the national legislation upon their first detection in Germany in 2016 and 2017, respectively. Although the highly potent Cumyl-PEGACLONE was initially considered as a relatively safe SCRA, in contrast to 5F-Cumyl-PEGACLONE, both compounds have already been linked to several fatalities according to the database of UNODC. (5F)-Cumyl-PEGACLONE is now scheduled in most countries, albeit with differing regulatory statuses regarding the compounds’ isomers; in Singapore the n-propylphenyl isomers are listed as scheduled compounds as well, while this is not the case for the ethylbenzyl isomers. Methods: In this study, the CB1 receptor activation potential of (5F)-Cumyl-PEGACLONE, together with two newly synthesized structural isomers (Cumyl-PEGACLONE ethylbenzyl isomer and n-propylphenyl isomer), was assessed using two different in vitro receptor-proximal bio-assays, monitoring the recruitment of either β-arrestin2 or a modified G protein (mini-Gi2) to the activated CB1 receptor. Results: Both in terms of potency and relative efficacy, Cumyl-PEGACLONE and 5F-Cumyl-PEGACLONE were found to exert strong CB1 activation, with sub-nanomolar EC50 values, and efficacy values exceeding those of the reference agonist JWH-018 >3 fold (β-arrestin2 assay) or almost 2-fold (mini-Gi2 assay). Importantly, a postulated difference in the activity of 5F-Cumyl-PEGACLONE and the non-fluorinated analogue could not be observed here, as both compounds were found to be equally active at the CB1 receptor in these in vitro bioassays. The ethylbenzyl and n-propylphenyl isomers showed strongly reduced CB1 activity (EC50 values >100 nM; efficacy <40% relative to JWH-018), which is hypothesized to originate from steric hindrance in the ligand binding pocket. None of the evaluated compounds showed significant biased agonism towards one of both signaling pathways. Conclusions: The functional assays applied here allowed us to demonstrate that 5-fluorination of Cumyl-PEGACLONE is not necessarily linked to an intrinsically higher CB1 activation potential, and that the ethylbenzyl and n-propylphenyl isomers yield a strongly reduced CB1 activation, with similar activity profiles for both isomers.

Synthetic Cannabinoids in hair – Prevalence in driver’s license regranting in Germany, March – September 2020
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Background: The use of Synthetic Cannabinoids (SC) has increased during the last decade. Additionally, the structural variety of this class expands continuously in order to circumvent generic laws like the German New Psychoactive Substances Act (NpSG). When sanctioned for driving under the influence of drugs (DUID) the driver’s license may be suspended. In order to regain the withdrawn license, drug abstinence must be proven by negative hair and/or urine samples

Emerging Drug Trends in the United States
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The abuse of analgesic opioids: a clinical case study
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Background: The abuse of analgesic opioids is a diffuse problem in the United States and in many other countries, including Italy, where cases of overdose and death are increasing. The aim of this presentation is to describe a clinical case study of a 39-year-old man. Methods: He was diagnosed with neoplastic lesion on the knee about 20 years ago and consequently prescribed oxycodone to reduce the pain. He abused the substance, while he was also suffering from paranoid schizophrenia and became dependent. He approached our Addiction Service, where he was prescribed methadone as a replacement therapy. Contact was made with his general practitioner and his previous psychiatrist in order

covering an observation period of at least 6 until up to 12 consecutive months, depending on the offense’s severity.
Methods: In this study, 2995 hair samples from 2693 individuals were analysed for “traditional” drugs of abuse as well as SC (qualitatively) during the period of March to September 2020. In this study only requests for the aforementioned abstinence control program with at least two successive samplings were considered. All sample details were anonymized before SC testing in order to prevent identification. Hair samples were decontaminated, extracted overnight and analysed by LC-MS/MS. THC was derivatized prior to GC-MS/MS in negative-ion chemical ionization (NCI) mode. Additionally, all specimens positive for SC were analysed with LC-ESI-qTOF-MS in order to search for new SC. In this presentation only qualitative results are shown. External contamination could not completely be excluded. Therefore, SC positive samples are not regarded as consumption of SCs but contact with the substance. Results: Only 0.4 % of the analysed hair samples were requested to be tested for SCs, none of them were positive. During the designated time frame, 577 people finished the abstinence control program successfully. However, being negative for all “traditional” drugs, 3.3 % of them were still positive for SCs and a quarter had a known history of THC consumption. 44 % of the SC-positive samples contained only one SC, in one sample 13 different SCs were found. In the hair samples positive for SC, 5F-MDMB-PICA was detected in half of the cases. In addition, seven SCs not covered by the qualitative LC-MS/MS method were found by LC-ESI-qTOF-MS and confirmed by semi-quantitative LC-MS/MS. Conclusion: Hair samples from the majority of persons reobtaining their driving permit were negative for all “traditional” drugs of abuse during the observation period. Nevertheless, 3.3 % were still positive for SCs which are rarely requested for analysis. Our results show that screening for SCs (at least of the second hair sample) is recommended in all cases, not only in those where individuals have a known history of cannabis consumption.

A comprehensive analytical process from the identification of an NPS threat to systematic screening
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Background: Despite reports of stabilization in the NPS global situation the phenomenon is still in constant evolution. This work aims to tackle NPS screening requirements with a complete NPS process including an early warning system (EWS) and a dynamic LC-MS/MS detection method allowing rapid addition/removal of compounds with a minimal and efficient validation. Methods: The LC-MS/MS method at the centre of this process relies on a high throughput protein precipitation extraction also used for 144 other drugs. A volume of 100 µL of blood/urine is precipitated with 400 µL of a 7:3 (v:v) acetonitrile:acetone mixture. The supernatant is diluted with a solution of 1.5% formic acid and injected on an Agilent 1200/1260 HPLC coupled to a Sciex 5500 QTrap operated in positive electrospray ionization mode with multiple reaction monitoring (MRM). Validation was achieved according to AAFFS Standards Board guidelines using 88 extracted samples and included evaluation of interferences, carryover, limit of detection, sample extract stability, extraction recovery and process efficiency. New trends were identified by an EWS monitoring detection events inside and outside the laboratory. Results: Validation criteria were met for all 57 analytes. Limits of detection (LOD) ranged from 0.01 ng/mL to 20 ng/mL, and sample extract stability from less than 24h to six days. There was no significant carryover, except for cis-3-methyl norfentanyl and fualprazolam, no interferences from 144 other common compounds as well as in between NPS screened for. All pair of isomers except isobutyl nortenfentyl and butyl nortenfentyl were resolved by a combination of chromatographic separation and careful choice of MRM transitions. Complete integration of the method to the existing workflow allowed systematic screening without performing a supplementary extraction and use more sample volume. Of the 5 079 cases screened for NPS, 175 (3.4%) tested positive, most of them were drug-impaired driving (140 cases, 80%), with only 30 post-mortem cases (17%) and 5 sexual assault cases (3%). New synthetic benzo diazepines were detected in 165 cases (94%), novel synthetic opioids in 8 cases (5%), and both categories in 2 cases (1%). The implementation of this method yielded a four-fold increase in the positivity rate of NPS. Conclusions: This ISO 17025 accredited process has the ability to keep pace with emerging trends as it is integrated to the already implemented workflow and thus allow systematic screening of every case. The LC-MS/MS screening method at its centre is sensitive, specific and use a widely available instrument. This workflow clearly demonstrated its usefulness in our setting, yielding a four-fold increase in NPS detection prevalence but its fundamental concepts can be adapted to any analytical workflow using a nonspecific extraction process.
to better understand the case. Results: After constant clinical monitoring, the patient managed to recover well. Conclusions: We believe that sharing knowledge and working jointly with other professionals are essential to reduce the risk of abuse of analgesic opioids and improve the care of patients.

Fentanyl Related Compounds and Designer Benzodiazepines Analyzed by the DEA Laboratory System
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Background: 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 17 substances reported for the first time in CY2019. Other chemical classes identified during CY2019 include benzodiazepines, benzofurans, piperoxanes, and several other classes. Mid-year 2020 data was not available at the time of abstract submission. 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.

NPS Discovery: A Program for Early Detection of Emerging Trends in Novel Drugs in the United States
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Background: Early warning systems (EWS) have been demonstrated to be especially effective in accelerating the sharing of information about newly emergent substances, which is critical for developing new analytical methods, for monitoring their spread and proliferation, for identifying outbreaks of adverse events in vulnerable populations and for prioritizing resources for control or scheduling actions. We describe the development and deployment of NPS Discovery in the United States with a mission to create a national alert and intelligence infrastructure for information sharing about new drugs, their use, and trends linked to public health and public safety. Methods: NPS Discovery was founded in 2018 with a goal of coordinating and disseminating activities of the non-profit CFSRE. These activities include new substance identifications, metabolomics studies, analytical method development, outbreak investigations, and sharing of information. The model has evolved into five primary domains: Intelligence, Surveillance, Monitoring, Response, and Forecasting. Dissemination of vital NPS information is shared at each point in this process and report include new drug monographs, public health alerts, and quarterly trend reports. Results: Through NPS Discovery, the CFSRE reported initial US appearance of twenty novel substances in 2019 and 2020, including six new opioids. Since 2018, 78 novel substances have been identified and reported, including stimulants, opioids, opioid precursors, benzodiazepines, hallucinogens, and synthetic cannabinoids. Since 2018, eight quarterly trend reports have been released for synthetic cannabinoids, six for opioids, and most recently two for benzodiazepines and stimulants/hallucinogens. In addition, public health alerts were issued concerning isotonitazene, brophine, fluvalprazolam, etylone, and 4F-MDMB-BINACA, as well as an outbreak involving synthetic cannabinoid/fentanyl combinations. During this period, CFSRE scientists also issued 20 peer reviewed publications describing analytical methods, metabolomics, adverse events, NPS related fatalities, and drug trends. These work products were ultimately distributed to an electronic mailing list of governmental, analytical, clinical, law enforcement, regulatory and harm reduction stakeholders. Conclusions: NPS Discovery has developed a comprehensive surveillance program offering the opportunity for international integration into a global monitoring system for NPS drug emergence and trends. This model provides opportunities for early detection, rapid response in scheduling, and preparedness by first responders and social services.

Elaboration of generic legislation for phenethylamines in Brazil
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Background: The New Psychoactive Substances (NPS) market is very dynamic, requiring new legislative approaches to tackle the drug problem. In addition to individual listings, in 2016, Brazil started adopting generic legislation for NPS. In 2019, continuing this strategy, Brazil adopted the generic legislation for the group of the phenethylamines, class of substances with documented psychoactive and stimulant effects that represents significant amount of seizures in Brazil. Methods: The generic legislation proposals were developed by an interinstitutional Working Group comprised of Anvisa and Ministry of Justice and Public Security. It was based mainly in scientific publications about structure-activity relationship, international legislation and materials from United Nations. The exchange of information and experiences
between different areas (sanitary regulation, forensic, law enforcement and drug police) was fundamental. Results: In the generic system, drug control measures foreseen under the individual listing system are extended to a defined group of substances, controlling large groups of molecules, without the need to list them individually. This includes substances that have not yet been reported, potentially preventing their emergence. It is especially efficient for NPS, since small structural changes to already controlled molecules are common in order to circumvent the control measures imposed on the original molecules. For the phenethylamine class, the Group proposed two main structures: 1-phenylethanol-2-amine and 1-phenylpropan-2-amine. Any substances falling within the structural classes described have been banned, with the exception of approved medicines. Conclusions: The NPS market continue to shift and diversify at alarming speed, posing a significant risk to public health and a challenge to drug policy. The adoption of generic legislation represents an evolution in the control system and an important strategy in the fight against the appearance and misuse of NPS. In Brazil, the integrated, joint and comprehensive work made possible by the interinstitutional Working Group has been shown to be extremely positive in that it allows combating the drug problem on several fronts.

**The pharmacometabolomic approach in the investigation of pharmacological effects of physiologically active substances using Danio rerio model**

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Background: Currently, there is an emerging amount of the so-called novel psychoactive substances, which are the newly synthesized drugs that mimic mind-altering drugs of abuse. Regarding this fact, the need for high-throughput models of drug screening is rising, and one of the promising models is zebrafish (Danio rerio). Zebrafish is useful for the studies of drug effects due to a large amount of physiological and genetic similarities to humans, high reproducibility, and relatively low maintenance cost. The main goal of our study was to identify the possibility of using Danio rerio for high-throughput screening of potential drugs of abuse to identify the psychoactive properties of drugs based on a targeted metabolomics approach. In this study, the pharmacometabolomic assessment was made to investigate the neurotransmitters profiles of zebrafish induced by diazepam, a drug of benzodiazepines family, and 5F-APINAC, a novel synthetic cannabinoid.

Methods: Twenty zebrafish eggs were placed in each well of 6-well plates and were exposed to a wide range of concentrations of diazepam (0.8 – 160 μg/L), and 5F-APINAC (0.001 – 10 μM) and each repeat of the experiment had a control group. Zebrafish were exposed to the chemicals for a different amount of time – from 2nd to 6th day post fertilization (“chronic” exposure) and for 2.5 hours for diazepam or 4 hours for 5F-APINAC at the 6th day post fertilization (“acute” exposure). 36 metabolites related to neurotransmitters metabolism were measured using UPLC-MS/MS approach. Results: Acute exposures. Alterations were found in serotonin, kynurenine pathways, dopaminergic/adrenergic system metabolites, and other metabolites related to neurotransmitters metabolism, such as aspartic acid metabolism, glutamic acid metabolism, GABA innervation system, acetylcholine system. Statistical differences were found in the concentrations of metabolites related to neurotransmitters, such as biotinidin and citrulline after both diazepam and 5F-APINAC exposures. Chronic exposures. Serotonin, kynurenine pathways, dopaminergic/adrenergic system metabolites, and other metabolites related to neurotransmitters metabolism, such as aspartic acid metabolism, glutamic acid metabolism, GABA innervation system, acetylcholine system were altered. Statistical differences were found in the concentrations of metabolites related to neurotransmitters, such as biotinidin and citrulline after both diazepam and 5F-APINAC exposures. Conclusions: In this comparative study, zebrafish have shown to have the potential to be used as a high-throughput screening model for the investigation of the effects of potentially psychoactive drugs.

**Club drugs and induced psychopathology**

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Background: The knowledge of health professionals on the psychopathological manifestations associated with substance intake is still scarce and fragmentary. Induced psychotic experiences are frequent, especially with some specific clusters of substances, like stimulants and psychedelics. Despite this, a deep knowledge of their clinical and psychopathological manifestations is lacking, as well as the differential diagnosis with primary psychotic experiences. Methods: a review of the most relevant studies about this issue will be reported. Some original data about the use of NPS in the island of Ibiza will be presented and discussed. Results: The lysergic psychosis is a construct that may be pivotal in the understanding of phenomena induced by substances in general, not only by lysergic hallucinogens. This term has its roots in the definition made by Hellpach and, most of all, in Bonhoeffer’s hexogen model, in which an external noxa may determine and directly influence the development of a full psychosis. Trying to explain in details what happens when an exogenous lysergic psychosis is emerging, we need to characterize it as a clear egodystonic experience, in which the subject perceives the presence of a “foreign body” in his own mind. The thinking Ego can feel and observe it as an uncommon experience, out of control, enriched by hallucinations (mainly visual and kinesthetic), delusional perceptions, and, in some cases, structured but confined delusional thoughts. The Ego is still aware and in charge of its role, and usually tries
to stem and contain the overflowing psychoma. However, taking into account its repetitive nature, the high dosages and the long-lasting pharmacokinetic properties of novel compounds, a new scenario may be hypothesized. If unusual thoughts or deviant perceptions become permanent, the capacity to deflate them is reduced to be more and more inadequate. The thinking Ego may not be able to counteract the psychoma anymore, and the latter can invade the functioning part of one’s mind, becoming fully pervasive. This could be the beginning of a psychotic experience, an “epidemic” diffusion inside the brain. Conclusions: The onset of psychiatric symptoms after the use of potent and highly rewarding drugs appears to be the norm, especially on the long term. These episodes, sometimes with clear psychotic features, are often reversible; however, when the use is frequent, persistent, and at high dosages, the onset of full and long-lasting disorders is commonly observed. The model of hexogen psychosis may help to understand what happens during and after the intake of these substances. More clinical studies are needed in order to clarify these aspects and the importance of the hexogen model, not only in the perspective of toxic psychosis.

The Rise of Flualprazolam in DUID, Sexual Assaults and Postmortem Toxicology Cases in Orange County, CA, USA

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Background: Flualprazolam has over taken etizolam as the most common novel psychoactive benzodiazepine in Orange County, CA. The number of flualprazolam cases has quickly increased with only seven cases in 2018 (starting in August), then 123 in 2019 and 119 in the first five months of 2020. Due to this increase, the Orange County Crime Lab decided to add flualprazolam to a benzodiazepine by LCMSMS quantitative method in order to report concentrations for ante-mortem and postmortem toxicology. The objective of this presentation will be to present flualprazolam concentrations for driving under the influence of drug, drug facilitated sexual assaults and postmortem cases in blood and tissue samples when available. Methods: Initially, 50 µL of Alprazolam-d5 (1000 ng/mL) was added to 0.25 mL of blood, urine or vitreous humor or 0.25 g of liver homogenate, brain homogenate, or gastric content homogenate. The protein was precipitated out of the sample with 0.75 mL of cold acetonitrile. The supernatant was then aspirated and dispensed on DPX-WAX tips three times, holding it on the tips for approximately 15 seconds with each aspiration. The final eluant was diluted with mobile phase and injected onto a Waters Acquity UPLC coupled to a Waters TQ-S triple quadrupole mass spectrometer utilizing positive electrospray ionization in multiple reaction monitoring mode. A Waters BEH C18 1.7 µm column (2.1 x 100 mm) held at 40°C with a gradient mobile phase of 0.1% formic acid in water and 0.1% formic acid in acetonitrile at 0.4 mL/min was used for chromatographic separation. A quadratic curve, weight 1/x2, was used for the range of 4 – 256 ng/mL with two control samples at 100 and 10 ng/mL. Results: For ante-mortem cases the average flualprazolam concentration is 22.56 ng/mL (median = 15.26 ng/mL) with a range of 4.03 – 133 ng/mL. For that same time frame, postmortem cases had an average concentration of 17.58 ng/mL (median = 9.59 ng/mL) and a range of 4.82 – 48.03 ng/mL. The flualprazolam tissue distribution for the postmortem cases varied greatly, but flualprazolam was detected in all matrices. THC and its metabolites along with other benzodiazepines (prescribed and non-USA prescribed) were the most common drugs found in conjunction with flualprazolam. Conclusions: With the data collected from this method a more thorough comparison to DRE findings, driving patterns and autopsy findings can be examined. Unfortunately, as with most other drugs, there are very few flualprazolam only cases. These few cases will be discussed at the end of the presentation.

A timeline of the co-detection of 4-Fluoroamphetamine and 25C-NBOMe in Australia

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Not available

A Sentinel Population: The public health benefits of monitoring enhanced body builders

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Background: There is heightened recognition of the public health implications of anabolic androgenic steroids (AAS) for the use of image and performance enhancement; with increasing evidence of their long-term negative health impacts, the hazards associated with their administration (often via injection), and the variability and unpredictability of their contents. In order to optimise the effects of these drugs, together with strict dietary and training regimes, AAS users typically supplement their use with an expansive and continually evolving range of ancillary drugs. The discovery and subsequent adoption of these drugs by the broader AAS user population is largely dependent upon a minority of social influencers within the bodybuilding community. Pioneering enhanced bodybuilders who self-experiment with a diverse range of image and performance enhancing drugs (IPEDs) and ancillary drugs have been the forerunners in the development of an underground user-led literature, online discussion forums, and were early adapters of internet-facilitated drug markets. Yet the impact of their self-experimentations extends well beyond the enhanced bodybuilding community, particularly in their use of ancillary drugs. Most significantly has been their role in the diffusion of various enhancement and
psychoactive drugs to the wider population. Methods: Using the theoretical framework of the 'diffusion of innovation' we consider the role that pioneering enhanced bodybuilders have played in the diffusion of various enhancement and psychoactive drugs to the wider population through a focus on three substances –dinitrophenol (DNP), melanotan II and gamma-hydroxybutyrate (GHB). Results: With an increasing range of drugs used by bodybuilders, coupled with an expansion in the use of online forums and online platforms to purchase pharmacological and new psychoactive drugs, we anticipate this trend of diffusion amongst the wider population will continue to flourish. Conclusions: Therefore, we highlight the need for policy makers to monitor emergent trends, not only in the general AAS population but particularly amongst enhanced bodybuilders.

‘Keeping Control’: The ethics of prosecuting substance abuse offenses in the COVID pandemic
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Not available

Metabolism study of N-methyl 2-amino Indane (NM2AI) and determination of metabolites in biological samples by LC-HRMS
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Background: Since the widespread diffusion of NPS, forensic laboratories are required to identify new drugs and their metabolites for which information or reference standards are lacking. Their identification in biological samples is very challenging, since the concentrations are extremely low, and they may be present mainly or solely as metabolites. As their metabolic pathways are often unknown, metabolism studies are necessary. Aminoindanes represent a relatively new generation of NPS. In recent years, these drugs have been increasingly reported to the UNODC Early Warning Advisory. None of them is currently under international control. Very little is known about their acute behavioural, toxic effects and metabolism. Methods: We performed a study on N-methyl-2-aminoindane (NM2AI) metabolism in silico and in vivo, to identify the main metabolites for biological samples screening. In silico metabolism prediction of NM2AI was performed using MetaSite software. The metabolites presence was verified in vivo (mice’s blood, urine, and hair) after NM2AI administration. Samples were analysed by liquid chromatography–high-resolution mass spectrometry (LC-HRMS) with a benchtop Orbitrap Exactive. We subsequently evaluated the agreement between software prediction and experimental results in biological samples. Results: LC-HRMS analysis identified seven main metabolites in the urine. They were identified, by their accurate masses and fragmentation patterns, as: 2-aminoindane (2AI), two hydroxy-2AI and four hydroxy-NM2AI; one of the hydroxy-NM2AI and one of the hydroxy-2AI also underwent conjugation. The study demonstrated the good concordance between in silico and in vivo results. The most probable metabolic products predicted by the software (2AI, two hydroxylated metabolites, and hydroxy-2AI) were found in authentic mouse urine; NM2AI and 2AI were also detected by LC-HRMS in the hair and blood samples. Based on these findings, we developed an LC-HRMS method for the screening of NM2AI and metabolites in urine, blood and hair samples. This can be of primary effectiveness to uncover the abuse of NM2AI and related possible intoxications. Conclusions: NM2AI was extensively metabolized; seven metabolites were identified in urine, marking the importance of metabolite identification for documenting NM2AI intake. In any case, the parent compound was always present at high concentrations. The present study marks one of the first developments of analytical methods for clinical, forensic and epidemiological investigations of NM2AI and metabolites in different bodily specimens. The identification of new or neglected compounds can be useful for the improvement of the Early Warning Systems, allowing the identification of NPS, whose use and toxicity, otherwise, would be underestimated.

Monitoring Synthetic Cannabinoid Trends in the United States
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Background: Since 2008, the synthetic cannabinoid illicit drug market has continued to change and evolve with one or more major new drugs being identified in the United States on a quarterly basis. These synthetic cannabinoids are subsequently identified in emergency room patients, impaired drivers, death investigations, and high-profile intoxications. Understanding the scale and scope of these events requires comprehensive analytical testing, which is frequently lacking due to the rate with which new compounds appear. From a laboratory standpoint, the challenges of remaining current with synthetic cannabinoids stem from the diversity of compounds in the class; the large number of analogues, configurations, and isomer; and the delays in availability of analytical standards to confirm presence in biological samples. Methods: In order to stay current with changing landscape of synthetic cannabinoids, a novel approach using both biological samples and sample extracts that had previously be tested were analysed using a comprehensive non-targeted data acquisition method by high resolution liquid chromatography time-of-flight mass spectrometry (LC-QTOF). The library was regularly updated with emerging synthetic cannabinoids and their metabolites as they became available and
ultimately resulted in a database with 288 synthetic cannabinoid parent compounds, metabolites, and internal standards. Acquired data was regularly reprocessed against the updated library to determine retrospectively the date of first appearance and spread of new compounds as they appear on the drug market. Results: Over the course of 15 months, different synthetic cannabinoid related analytes were detected (25 parent compounds and 13 metabolites) which correlated to at least 27 unique synthetic cannabinoids. 5F-MDMB-PICA was the most prevalent analyte identified during this funded research, followed by 5F-MDMB-PINACA, MMB-FUBINACA, and 4F-MDMB-BINACA. Through the second quarter of 2020, 5F-MDMB-PICA continued to be the most frequently detected synthetic cannabinoid. MDMB-4en-PINACA was identified for the first time in the first quarter of 2020 and 5F-EMB-PICA in the second quarter. Conclusions: In the United States, synthetic cannabinoids pose significant challenges for public health and public safety agencies. New synthetic analogues that target endogenous cannabinoid receptors continue to appear on recreation drug markets, sometimes increasing in potency and toxicity in comparison to previous generations. Using this approach, we developed a model that allowed for the timely identification and dissemination of newly identified synthetic cannabinoids. Current trends suggest that new synthetic cannabinoids appear on a monthly basis, but typically only one (or two) analyte(s) will proliferate and dominate the market for roughly one year in time. Continued vigilance efforts must be maintained to stay current with evolving market.

The NIST Fentanyl Classifier

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Background: Fentanyl is a highly addictive synthetic narcotic originally developed for treating severe pain. The growth in the abuse of fentanyl, fuelled in part by “designer” fentanyl analogues, has led to an unprecedented rise in overdose deaths. The large and rapidly evolving number of these drugs presents a challenge in chemical identification. This presentation will describe the application of mass spectral similarity mapping towards the classification of designer fentanyl analogues. Methods: Mass spectral similarity mapping is a natural extension to traditional mass spectral similarity searching. In both processes a query mass spectrum of the analyte is searched against a library of reference spectra. Whereas a traditional similarity search provides an analyst with a hit list -- a high confidence way of identifying an analyte with a replicate spectrum in the library -- similarity mapping provides a map that is informative even for analytes that are not in the reference library such as new designer drugs. A mass spectra library that included fentanyl and “Type I” fentanyl analogues was collected, where “Type” indicates the number of modification sites by which an analogue differs from the standard fentanyl scaffold. Mass spectral similarity maps can be generated using query spectra and the fentanyl library. These maps may be scrutinized to classify the query based on the likely site of modification and, in some instances, propose a complete structure for the query. An open-source implementation of mass spectral similarity mapping applied to fentanyl analogues, referred to as the NIST Fentanyl Classifier, is available at https://github.com/asm3-nist/fentanylClassifier. Results: The NIST Fentanyl Classifier (NFC) was tested using several example spectra, including replicate spectra of fentanyl, replicate spectra of the Type I fentanyl analogues contained in the library, spectra of Type I fentanyl analogues not represented in the library, spectra of Type II analogues, and spectra of non-fentanyl compounds. As expected, the NFC correctly localized modification sites for fentanyl analogues that differ from fentanyl by modification on at most two sites (i.e. Type I and Type II fentanyl analogues). In cases where the query was a Type II fentanyl analogue with “composing cognates” contained in the library, the NFC was able to accurately identify the query short of positional isomers. Conclusions: The NIST Fentanyl Classifier is a new tool that applies mass spectral similarity mapping to the analysis of fentanyl and fentanyl analogues. It has demonstrated applicability and limitations, both of which will be described in this presentation.

NPSfinder: a systematic tool to identify novel psychoactive substances (NPS) by crawling the pro drug/’psychoanaut’ websites

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Background: The online market for new psychoactive substances is developing much faster than academic research. In addition to the traditional websites, cryptomarkets have also recently been developed. These are anonymous sites that operate on the so-called “darkweb” and are accessible only through specially configured browsers. Vulnerable subjects, including children/adolescents and patients with psychiatric illnesses, may be exposed to web pages that provide opportunities to purchase drugs and/or drug information. “NPSfinder” is a “crawling/navigating” software that was designed by the Rome (I)-based Drug Surveillance Unit and the informatics’ Damicom team. Methods: NPSfinder allows to map on a 24/7 basis the large variety of psychoactive molecules mentioned within the range of major online psychoanaut web sites/fora. NPSfinder is a tool able to extrapolate a range of information regarding NPS, including: chemical and street names; chemical formula and IUPAC nomenclature; three-dimensional image; and, when available, anecdotally reported clinical/psychoactive effects. These data are automatically stored into an online database, which is Located
within firewall protected, highly secure, and consistently performing servers. NPSfinder’s goal is to map the entire number of existing psychoactive molecules and smartdrugs on the internet. Results: After about 18 months of operation, the number of substances identified by the web crawler activities was 5922; of these molecules, 4192 different molecules were included in the database and 1730 (29.2%) of the remaining molecules were false positives/duplicates. Hence, one could provisionally reckon that the online/psychonaut web fora NPS scenario might include in the region of 4000 different molecules. The researchers assigned each molecule to its drug class, with the following classes having been identified: aminoindanes, synthetic cannabinoids, cathinone derivatives, psychedelic phenethylamines, synthetic opioids, novel stimulants, tryptamine derivatives, phencyclidine-like dissociatives, piperazines, GABA-A/B receptor agonists, a range of prescribed medications, psychoactive plants/herbs, and a large series of image-and performance-enhancing drugs (IPEDs). The most popular NPS mentioned in the psychonauts’ fora included: phenethylamines (30.1%); synthetic cannabimimetics (29.8%); synthetic opioids (10.2%); synthetic cathinones (4.1%), designer benzodiazepines and other GABAergic drugs (4.1%); and prescribed drugs (3.8%). Conclusions: NPSfinder is a useful tool to facilitate the process of early recognition, it allows to follow a phenomenon that otherwise would be difficult to monitor constantly, due to the increasingly dissemination of new substances online and the instability of information sources. The early identification of NPS has crucial medico-legal implications and will also bring significant benefits to the daily clinical practice of physicians working in both emergency departments and general psychiatry.

**Carfentanil advertised on the darknet: potential scam or alarming public health threat?**

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Background: In an age of global insecurity, highly potent synthetic drugs have increasingly been used to harm others. Their online advertisement and sale are facilitated by surface web, darknet markets and social media fuelling various types of new criminal activities and their growth in sophistication. This study presents a systematic analysis of the darknet sale of one of the most potent synthetic opioids: Carfentanil. With an equianalgesic potency of 10.000 times a unit of morphine, its toxicity is comparable to traditional nerve agents, and it has been previously used as a chemical weapon, causing human fatalities. Methods: Digital trace data was collected retrospectively from all the darknet marketplaces, which have been active in the past five years. Data on vendors offering Carfentanil on Agartha, Empire and Yakuza marketplaces were analysed with regard to items sold and sellers’ features as these were the only active markets at the time of search. Searches were carried out in the English language only. Results: 63 different Cartfentanil vendors operating on 19 darknet marketplaces were identified. Contacts and payments were facilitated with end-to-end encryption messaging mobile applications and content-expiring messages. Although it is known that Agartha is a scam market, and no operative sellers were found on Yakuza, several sellers promoting Carfentanil sales were active in Empire marketplace, with a number of transaction ranging from 4 to 1223. Conclusions: The availability of highly potent drugs such as Carfentanil on the darknet requires the development of innovative scientific methods and tools able to monitor and predict such new threats, while informing policymaking and protecting the health and the security of citizens.

**Mapping NPS policy in the EU: legal frameworks, healthcare provision and outcomes**

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Background: The rise in NPS trade and use as well as the lack of information concerning their risk to drug users’ health pose serious challenges to European public health authorities. The aim of this contribution is to present a general assessment of NPS-related policies implemented by ten European countries through the lens of legal epidemiology. Methods: A mapping review of drug-related legal instruments and policy documents was performed. It was followed by comparative analysis aimed to identify main features of NPS-related policies implemented across Europe (Belgium, Czech Republic, England, France, Germany, Ireland, Poland, Portugal, The Netherlands, Scotland). The conceptual framework used for the analysis encompasses law philosophical principles, institutional arrangements and the specificity of policies. Law’s intended and incidental effects on health was assessed based on countries drug-related health outcomes. Results: The countries under study can be placed in a wide spectrum according to the general principles that define their drug policy. Those who have implemented specific legal responses to NPS are based on different regulatory models, from blanket bans to generic and individual classification of substances. Besides, the implementation of harm reduction interventions (awareness campaigns, drug checking services and monitoring systems) are among the public health responses provided by some countries regardless the legal status of NPS. However, prevalence of NPS use seems to be more linked to general availability of psychoactive substances (including illicit drugs) rather than to law enforcement measures, while punitive measures seem to have a negative impact on NPS-related intoxications and deaths. Conclusions: There is still limited development towards harmonisation of national drug policies within Europe, particularly with regard to NPS. Although harm reduction interventions seem to have a positive impact on preventing intoxications and managing
The detection of synthetic cannabinoid receptor agonists in infused papers from prisons in a constantly evolving illicit market

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Background: In Scotland, synthetic cannabinoid receptor agonists (SCRAs) are almost exclusively used in prisons, with little use in the wider community. The majority of SCRAs are infused into paper and card and enter the prison via the mail system, where they are vaped. Scottish prisons can therefore act as an early warning system for the emergence of new SCRA compounds and modes of use. Qualitative and quantitative laboratory-based methods were developed for the detection of SCRAs on infused paper and an evaluation of field-deployed ion trap mobility spectrometer (ITMS) instruments used in prisons to screen SCRA in incoming mail was carried out. The utility of near real-time screening of samples seized from Scottish prisons for monitoring and intelligence purposes was explored.

Methods: Methods for the qualitative and quantitative analysis of SCRA infused papers were developed using gas chromatography-mass spectrometry (GC-MS) with qualitative confirmation by ultra-high-pressure liquid chromatography (UPLC-PDA-QToF-MS) and applied to non-judicial seized paper samples from Scottish prisons. 392 non-judicial paper samples seized from four Scottish prisons between June 2018 and December 2019 were examined. Additional data on the SCRA detected in samples seized between September 2019 and August 2020 showing the continuing evolution of the SCRA market in prisons will also be presented.

Results: From June 2018 to December 2019, 392 seized paper samples from four Scottish prisons suspected of being infused with controlled substances were analysed and 46% were positive for at least one SCRA, concentrations ranging from <0.05-1.17 mg/cm2 paper. After September 2019, the prevalence of MDMB-4en-PINACA increased and by March 2020 was the most commonly detected SCRA in Scottish prisons. Our evaluation of two ITMSTM systems confirmed that both instruments were effective for the testing of prison samples with up to 95% agreement with laboratory-based GC-MS analysis.

Conclusions: A method for the qualitative and quantitative analysis of SCRA in paper was developed, validated, and successfully applied to a large number of samples seized from Scottish prisons. This has provided near real-time information of the emergence of new SCRAs in the prison system, reflecting international market trends. The Rapiscan Itemiser® 3E and Itemiser® 4DN ion trap mobility spectrometerTM (ITMSTM) systems were found to be effective for the rapid in-field presumptive detection of SCRA infused papers and used effectively, will help to reduce the supply of SCRAs in the prisons. Future work will focus on the training of operational staff to indicate the emergence of new SCRAs on the market and on the development of methods to predict instrument response for compounds yet to emerge.

Functional characterization of hallucinogenic NPS using different 5-HT2AR bioassays

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Background: Hallucinogenic new psychoactive substances (NPS) continue to comprise a large portion of newly detected substances. While being a structurally divergent group, they share their main pharmacological mechanism: the activation of the serotonin 2A receptor (5-HT2AR), which is responsible for the typical hallucinogenic effects. Due to the rapid pace at which these substances emerge on the drug market, they are-and often remain-poorly characterized in terms of potency and efficacy. Furthermore, the structure-activity relationship of these compounds and their mechanisms on a molecular level remain to be elucidated further. Methods: For the characterization of compounds, new bioassays monitoring the activation of 5-HT2AR by hallucinogenic NPS were developed, to complement the previously reported transient \( \beta \)arr2 recruitment assay. More specifically, a stable cell system of the latter was generated, for reduced experimental variability. Furthermore, also a transient system employing an engineered miniGaq protein, was optimized, which, together with the \( \beta \)arr2 assay, enables the assessment of signaling bias for hallucinogenic compounds at this receptor. All bioassays were based on a luminescent readout, following functional complementation of a split nanoluciferase (NanoBiT technology).

Results: During the series of experiments, several endpoints were assessed, of which the results will be presented: First of all, the performance of the developed \( \beta \)arr2 stable cell line was compared to that of the previously developed \( \beta \)arr2 transient bioassay, finding a high comparability of the results. Secondly, a panel of thirty structurally diverse compounds was tested in the new stable \( \beta \)arr2 bioassay. This enabled the establishment of a structure-activity relationship between, among others, 2C, NBOMe, NBOH and amphetamine-like compounds (DOx). As a third outcome, the simultaneous use of the transient bioassays for \( \beta \)arr2 and miniGaq recruitment, gives an estimate of the signaling bias that could be inherent
to certain compounds. For this purpose, a set of 13 compounds was subjected to the two bioassays, identifying 4 significantly biased agonists. Conclusions: In conclusion, the reported bioassays and their use for the assessment of characteristics of hallucinogenic compounds, provide an estimate on the structure-activity relationships between these compounds, their potencies and efficacies and their characteristics on a molecular level. Furthermore, the results obtained with the stable βarr2 cell system, show significant correlation with estimated common dose estimates.

Is Kratom (Mitragyna speciosa) a safe medicinal aid? A Netnographic Analysis of Experiences and Comments on YouTube™

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Background: Kratom (Mitragyna speciosa) is a plant native to Southeast Asia with history of traditional medicinal use. The aim of this study was to investigate its safety and therapeutic benefits as reported on YouTube videos. Methods: Data from the most viewed videos were extracted via the machine learning algorithms generated by the YouTube Application Programming Interface (API). Results: A total of 500 videos with 19,478,180 views and 134,863 comments emerged from this data scrape. 12 out of the 16 most viewed videos were manually processed and selected for inductive thematic analysis. Kratom was described here as an aid to self-treat various health conditions, such as opioid dependence/addiction (83.4%), pain (75%), anxiety (67%) and depression (42%), substance use problems (42%) as well as for energy boosting (50%), mood elevation (25%) and nootropic effects (25%). Although most of the described experiences were positive (58%), side-effects such as dependence and withdrawal (50%), nausea (42%), loss of appetite (25%), sedation (25%), loss of motivation (16.7%), headache (16.7%), drowsiness (16.7%), dry mouth and frequent urination (16.7%) were also reported and linked in 25% of the cases to chronic ingestions. Conclusions: The current YouTube videos provide promising insights about the medicinal potential of Kratom use. Further controlled clinical studies are needed to better determine the therapeutic efficacy and long-term perils of Kratom use.

Facing ever-changing trends: prevalence and patterns of New Psychoactive Substances use in the young population of Kazakhstan

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Background: The strong association between the young age and NPS use has received increased attention in various studies. As for 2012, the Kazakhstan cluster-based survey in educating youth revealed that 15.8% of young people have used drugs in their lifetime. The national assessment of NPS prevalence among hospital-based drug users demonstrated a stable upward trend over the three-year period (2016-2018): from 3.91% to 10.01% of all drug dependence-related hospitalizations in the country. Just above a quarter of the subjects were under 25. The aim of this study was, therefore, to obtain the initial insights into the socio-demographic determinants, patterns and correlates of NPS use in the young population of Kazakhstan. Methods: The cross-sectional study was conducted in 17 major cities across Kazakhstan in July 2019. The target population was young people aged 18-34. The quota sampling was based on the proportions within the given age group and its gender distribution. The sample size was set at 1500 respondents. Results: The proportion of those who used NPS at least on one occasion was 6.3% (5.2; 7.7), which ranged between different regions from 0 to 12.0% (7.5; 18.0). NPS users prevailed in men, aged 25-29. Up to 3.4% (2.6; 4.4) used NPS in the last 12 month, 2.4% (1.7; 3.3) - in the last 30 days. Two thirds of the respondents inhaled synthetic cannabinoids, just above a half consumed synthetic cathinones with various routes of administration. Overall, 30% of NPS users bought drugs from dealers, the same proportion got NPS through social messengers (e.g. Telegram, VIpole). The youth reporting NPS use were also 1.5, 1.23 and 7 times more likely to use tobacco, alcohol and illegal drugs, respectively, compared to respondents who did not report NPS use. Conclusions: Compared to the other Central Asian countries, the prevalence of NPS use in the Kazakhstan young population is considerably higher and corresponds with an upward trend of NPS hospitalizations in drug treatment clinics. NPS in the context of polysubstance use pose a serious concern and should be monitored especially in the challenging time of pandemic changes.

How to maintain activities during a crisis period

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In this short video, Dr Olivier Rabin, Senior Executive Director for Sciences and International Partnerships at the World Anti-Doping Agency (WADA), will briefly describe how WADA reacted to the initial phase of the COVID-19 pandemic and quickly adapted its operations to protect the health of its personnel, to maintain its core activities and to provide...
guidance to the international anti-doping community. In particular the essential role played by rapid collection of reliable local and international information and objective opinions provided by internal and external experts to ensure solid decisions were taken by the Agency to guide our stakeholders and guarantee continuity of international activities in the early phase of the crisis.

**Profiling NPS in human clinical trials**

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New Psychoactive Substances (NPS) are non-scheduled drugs ("legal highs") that mimic the effects of traditional drugs of abuse. The unprecedented proliferation of NPS on the drug market threatens public health and challenges drug policy. Information on their clinical pharmacology and toxicity is in most cases very limited. Given the large number of new compounds released on the market each year a timely evaluation of NPS by current standards in ex-vivo and in-vivo animal models or expert evaluations is unfeasible. We recently created a machine learning algorithm employing the quantification of metabolomics in rats to predict the similarity of new drugs to classical ones of abuse. The model successfully predicted the pharmacological profile of a synthetic cannabinoid (JWH-018) as a cannabinoid-like drug and synthetic cathinone (mephedrone) as a MDMA-like psychostimulant. As a follow-up, we propose a targeted imaging-metabolomics approach in humans that provides fast classifications of NPS and their effects on brain function. A predictive model based on machine learning algorithms will use the quantification of neurotransmitters, endocannabinoids, steroid hormones and their metabolites in blood, functional connectivity in resting state brain networks and neurocognitive function to predict the similarity of a new drug to classical drugs of abuse. As a proof of concept, the model will be tested to forecast the impact of NPS on brain function based on their metabolome in blood. Our approach will support fast risk classification of NPS and benefit public health.

**Methadone as a possible cause of two healthy teenagers’ death**

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Background: There have been significant changes in adolescent consumption habits over the past fifteen years., especially due to the fact that new molecules have been synthesized, new devices created, and a number of products have increased in popularity. Methods: We report the case of two 15 and 16-year-old adolescents died during sleep after the assumption of a lethal unidentified mixture. The teenagers accidentally purchased methadone instead of codeine in order to prepare a Purple Drunk, a dangerous cocktail commonly used by teenagers, made popular by American rappers and social networks; it generally combines codeine-based cough syrup, antihistamines such as promethazine and soda. After taking the cocktail containing methadone, both boys experienced fatigue, dizziness, drowsiness and widespread nonspecific abdominal pain; these symptoms forced them to stop the football match they were playing with friends and to go home. After a few hours they died during sleep. Results: At the moment heart failure is considered the most likely cause of death but the autopsy did not detect congenital or acquired cardiac abnormalities in both patients. Currently, toxicological tests are in progress to clearly identify the specific components of that lethal mixture containing methadone responsible for both patients’ death.

**PIEDs and psychopathology: the clinical issues**

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Background: The presence of Performance and Image Enhancing Drugs (PIEDs) amongst general population and patients is arising, as described by the Keep Fit study of the University of Hertfordshire. During our activity in a Dual Diagnosis Unit located in the North East of Italy (Clinica Parco dei Tigli, Padova) the use of these supplements and products has been largely reported with some serious consequences regarding the psychopathological state and the risk of dependences. Methods: We collected some clinical cases where the use of PIEDs, the presence of classic substances (e.g. cocaine) and some psychopathological (like Exercise Addiction [EA] and Body Dysmorphic Disorder [BDD]) were assessed and treated. Results: In our clinical experience the presence of these compounds is essential for a correct diagnosis. PIEDs can mislead the treatment and must be identified by clinician in order to give some advice and correct information about PIEDs’ health risks. In particular the relation between PIEDs and some psychopathological issues (EA, BDD), as explored in the Keep Fit results, has been confirmed by clinical evidence. Conclusions: The use of PIEDs should be always explored both amongst general population and patients, because the health professional must a) provide correct information regarding each class of compounds and their side effects b) guide every intervention considering their presence and the psychopathological risk related to them.
Treatment Compliance among Incarcerated and Fined Amphetamine-Type-Stimulant (ATS) Users in a Community Supervision Programme in Malaysia: A Preliminary Study
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Background: People caught using amphetamine-type-stimulants (ATS) in Malaysia can either be incarcerated and subsequently be placed in a community supervision programme or if they can afford the fine, be directly sent for community supervision. We sought to determine if treatment compliance in the community supervision varied between the two groups. Methods: Eighty-five ATS users (59 previously incarcerated persons and 26 having only been fined) were recruited from a community supervision programme for this longitudinal study. The sample was largely male and Malay. Respondents were screened for drug use and assessed for six months. Results: We found that 54% of the incarcerated participants had dropped out of the supervision programme as compared to 23% of the fined participants. Also, by the end of the study period, 63% of the incarcerated participants had tested positive for drug use, relative to 30% of the fined participants. Furthermore, incarcerated participants had higher odds of having previously dropped out of a community supervision programme (OR: 4.34; 1.44-13.06; p<.006), as compared to their fined counterparts. Conclusions: ATS users with a history of incarceration had poorer treatment compliance, relative to their compereers who were not incarcerated and placed directly in a community supervision programme.

The Relationship between Amphetamine-Type Stimulant (ATS) Use and Violent Crime in Penang, Malaysia: Findings from a Preliminary Study
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Background: Although a few studies have described the association between amphetamine-type-stimulant (ATS) use and crime, the relationship still remains inadequately investigated. This study examines the link between the length of ATS use and violent crime, in a sample of ATS offenders detained in a police custody in Penang, Malaysia. Methods: One hundred and forty-nine offenders with current ATS use history were recruited for this cross-sectional study. All the study data was collected through face-to-face interviews with a semi-structured questionnaire. Results: The majority were males (93%, n=138/149), and the respondent’s mean age in the study was 36.19 years (SD=11.51). Sixty-two percent (n=93/149) had six-years ATS use history, and 60% used ATS >4 days per week (n=89/149). The majority used ATS for its stimulant/stamina effects, to overcome boredom and stress. Computation of unadjusted odds indicated that long-term ATS users had higher odds of physically assaulting their crime victims in the last six-months (OR: 2.34; 1.16-4.72; p<.016), used ATS before committing crime (OR: 2.87; 1.45-5.69; p<.002), and committed crime with the use of a dangerous weapon (OR: 3.35; 1.59-7.07; p<.001). However, these differences were not evident in multivariate logistic regression analysis. Conclusions: Our preliminary findings show that prolong ATS use serves as a risk factor for offending behaviours in Malaysia. Thus, targeted treatment interventions are needed in lieu of incarceration, to reform offenders with drug use problems.

Kratom (Mitragyna speciosa Korth.) Use among Opioid Users with Amphetamine-Type-Stimulant (ATS) Use History in Malaysia
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Background: Kratom (Mitragyna speciosa Korth.), an indigenous plant of Southeast Asia, has been traditionally used for its medicinal value, and as a cure or substitute for opioids/heroin in the Siamese-Malay states, despite the fact that it is not recognized as a legal medicine by the Malaysian National Pharmaceutical Regulatory Agency. Though kratom is reported to produce opioid and stimulant like-effects, studies of its use and perceived utility among opioid users who also use amphetamine-type-stimulants (ATS) remain limited. We investigated reasons for kratom use among opioid users with ATS use problems in Malaysia. Methods: A total of 427 opioid users with ATS use problems were recruited through convenience sampling from five Community Care and Rehabilitation Centres for this cross-sectional study. Survey responses were collected with a semi-structured questionnaire. Results: Of the total sample, 332 participants were kratom users and 95 were not. Among regular kratom users, the mean duration of kratom use was 6.9 years (SD=1.69). Most used kratom decoctions to suppress heroin withdrawal symptoms (77%). More than half used kratom as an ATS or heroin substitute (59%), while 58% used kratom to reduce heroin use alone. Multivariate analysis results show that long-term opioid users with ATS use problems had higher odds of using kratom for prolonged periods (p<.018), and as a substitute for ATS (p<.023). Short-term users were more likely to use kratom to suppress heroin withdrawal symptoms (p<.006). Conclusions: Opioid users with ATS use problems, commonly used kratom for long-term periods to reduce dependence from both types of these addictive illicit substances. There are currently no pharmacotherapies clinically indicated or approved for ATS use disorder treatment. Findings merit further exploration as polydrug users with ATS problems in this study reported ATS substitution with kratom.

Treatment Barriers Associated with Amphetamine-Type-Stimulant (ATS) Use in Malaysia
Background: Several published studies have already shown reluctance among amphetamine-type-stimulant (ATS) users to seek treatment. This study investigated the problem in Malaysia. Methods: 386 ATS users were recruited from five compulsory drug detention centres (CDDCs) for this cross-sectional study. The data were elicited through interviews and a semi-structured questionnaire. Results: The majority of ATS users were males (83%, n=321/386) and only 17% (n=65/386) were females. The commonly reported ATS treatment barriers include self-reliance in treating ATS use, fears of discrimination by the community, peer influence, lack of confidence in the community supervision program in aiding recovery, long waiting time for treatment, lack of family support, shame of staying in a treatment centre, difficulties in registering for treatment, and lack of desire to give up ATS use. Female ATS users had higher odds of reporting fears of community discrimination (OR: 1.80, 1.03-3.12, p<.037), peer influence (OR: 1.89, 1.10-3.25, p<.020), and long waiting time for treatment (OR: 2.74, 1.58-4.72, p<.000), as compared to male ATS users. Conclusions: Several important barriers inhibit ATS users from seeking treatment in Malaysia.

**Effects of External Influences on Synthetic Cannabinoid Trends in New Zealand, 2014 to 2020**


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**Detection of Synthetic Opioids, Synthetic Cathinones, and Synthetic Cannabinoids in Wastewater in the U.S. – A Promising Community Early Warning System**

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MAB-CHMINACA, methcathinone, 4-methyl pentedrone, 2-methyl-4’-(methylthio)-2-morpholinopropiophenone (MMP), 1-(3-chlorophenyl)piperazine (mCPP), and 5-(2-Aminopropyl)indole (5IT) were quantified. Methcathinone was the most frequently detected NPS (detection frequency, df = 100%) followed closely by the MMP and mCPP (df = 91%). The mass loading of methcathinone, mCPP, and 5-IT, using ammoniacal nitrogen-based population, were up to 21.1 ± 1.3 mg/d/1000 people, 15.0 ± 0.5 mg/d/1000 people, and 9.75 ± 2.72 mg/d/1000 people, respectively. Two synthetic cathinones (methcathinone and 4-methylpentedrone) and three other NPSs (4-ANPP, mCPP, and 4-methylamphetamine) were also quantified in wastewater indicate the prevalence of NPSs in Kentucky. This is the first study to determine the occurrence of NPSs including synthetic opioids, synthetic cannabinoids, synthetic cathinones, and piperazines in the U.S. communities and mass gatherings. Conclusions: Several NPSs can be detected and quantified in raw wastewater. The routine screening of several NPSs utilizing state-of-art analytical method can be used as an early warning system for the community prevalence of NPSs.

**Identification of 11 substances known as “smart drugs” in Japan**

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Background: In recent years, substances known as “smart drugs”, also known as “nootropics”, have been detected in Japan. The smart drugs have been used in the hope of enhancing human brain activity. These substances are easily imported and can be used without a prescription. However, the quality, effectiveness, and safety of these products have not been confirmed. In this study, 11 substances known as “smart drugs” were isolated and identified from the products sold as reagents in Japan. Methods: The 11 white powdery products in small plastic containers were obtained in Japan between October 2015 and November 2019. Although these products were sold as reagents on the internet, each container was labelled with the name of the substance considered to be a smart drug. The product was extracted with methanol, followed by filtration by centrifugal filter. The resultant solution was analysed by LC-ESI-MS and GC-EI-MS. The accurate mass spectrum of the target substance was measured by LC-QTOF-MS. Identification of the substances was performed by NMR (1H-NMR, 13C-NMR, HMOC, HMBC, H-H COSY). Results: As a result of the LC-ESI-MS and GC-EI-MS analyses of the 11 products, 11 substances were detected. Five of these substances were identified as Adrafinil, CRL-40,941, Fenozolone, Coluracetam and PRL-8-53 by comparing the data with those of the authentic substances. The other six unknown substances were analysed by the LC-QTOF-MS and NMR, and identified as N-Methyl-cyclazodone, N-(2-Cyanoethyl)phenethylamine (PEA-P), 1-Phenyl-2-propylaminopentane (PPAP), p-F-Deprenyl, Unifiram and Morphodrol. Each substance corresponded with the name labelled on the container. The analytical data of LC-ESI-MS, GC-EI-MS and NMR showed that each of these products consisted of almost a single substance. Of these, PEA-P is classified as a group of phenethylamines, and p-F-Deprenyl is an analogue of Deprenyl, developed as a drug for Parkinson’s Disease. Coluracetam is a substance developed as a drug for Alzheimer’s Disease. Adrafinil and CRL-40,941 are analogues of modafinil, a psychostimulant, used for the treatment of narcolepsy. Conclusions: Most of the substances detected in this study are analogues of pharmaceuticals. Since these pharmaceuticals have pharmacological effects on central nervous system, an excessive intake of these analogues may cause health problems.

**Prevalence and social-cognitive determinants of the use of Performance Enhancing Substances by Portuguese Gym-Goers**

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Background: The use of performance-enhancing substances (PES) occurs among gym goers, which is seen as a public health concern. The aim of this study is to investigate the extent of PES use and the social-cognitive determinants which influence the intention to use these substances among gym goers, according to the Theory of Planned Behaviour. Methods: A convenience sample of Portuguese gym goers (n = 453; 61.3% female; 38.7% male) completed an anonymous web-based survey about beliefs, attitudes, social influences and intentions towards use of PES as well as self-reported PES use. A two-step approach to maximum likelihood, structural equation modelling, multigroup analysis and t-test with the Welch correction for heteroskedastic variances were performed using IBM SPSS/AMOS 24.0. Results: 11.01% of the participants reported PES use. At the structural level, results support attitudes (β = 0.21; p < 0.001), beliefs (β = 0.35, p < 0.001) and subjective norms in predicting intentions to PES use in gym goers with subjective norms (β = 0.50; p < 0.001) being its strongest predictor. Moreover, results showed a significant association self-reported PES use and intentions to use (β = 0.66, pp < 0.001). The predictive model was invariant across gender; however, compared to males, females believed less in the performance enhancing effects of PES, were less prone to the influence of significant others and had weaker intentions to use these substances. Conclusion: Preventive interventions should focus on influencing subjective norms, alongside to beliefs and attitudes towards PES use as these variables influence the intention to use PES in this particular population.

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**The Growing Complexity of the Opioid Crisis**

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The current opioid crisis is a far-reaching drug and public health policy issue affecting several geographical regions. Since its appearance, efforts have been made both at the national and international level to develop integrated policy responses to address the crisis. Yet, despite some progress, the crisis continues to deepen in complexity with the emergence of a new generation of new psychoactive substances (NPS) with opioid effects, including substances belonging to chemical structural classes which were not significantly present on illicit drug markets previously. The presentation provides an overview of the multi-faceted opioid crisis and highlights major international and domestic policy responses to date. It presents selected key developments related to NPS with opioid effects, examines how these developments are influenced by existing control measures and outlines possible policy responses.

**Acute effects of a moderate dose of a synthetic cannabinoid (JWH-018): implications for psychosis**

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Background: Synthetic cannabinoids are being used for more than 10 years, but placebo-controlled studies into their effects are still scarce. SC’s are much more potent than natural cannabis, and as a result many survey’s report more psychological problem. In the current study we investigated the subjective drug response to an acute dose of the synthetic cannabinoid JWH-018 up until 4 hours after administration. Methods: In this placebo-controlled study, 24 healthy participants received a dose of 75µg/kg bodyweight JWH-018. Their subjective intoxication was recorded and reached a minimum level before the test day was continued. The acute effects on psychedelic symptoms, dissociative state and mood were measured with subjective rating scales (resp. Bowdle visual analogue scales, Clinician Administered Dissociative States Scales (CADSS) and Profile of Mood Scales (POMS)). Results: Data were analysed using a GLM Univariate ANOVA with Drug (placebo and JWH-018) as a within subject factor. The within factor Time was added in case a test was taken more than once on a test day. Subjective high was significantly increased after the JWH-018 administration and maximum subjective high (average 6.4cm) was reached 30 minutes after administration. After 4 hours the subjective score did no longer differ from baseline. The Bowdle scales External and Internal perception, and high and drowsiness were significantly increased after JWH-018 intake. JWH-018 significantly increased the CADSS scales amnesia, depersonalization, derealisation and total score. Fatigue, Confusion and Arousal scales of the POMS differed significantly from placebo and baseline after administration of JWH-018. Conclusions: It is concluded that JWH-018 is able to induce psychotomimetic effects in healthy participants with no history of mental illness.

**In vitro functional characterization of a panel of non-fentanyl opioid new psychoactive substances**

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Background: The landscape of new psychoactive substances (NPS) is constantly evolving, with new compounds entering the illicit drug market at a continuous pace. Of these, opioid NPS form a particular threat owing to their high potency and prevalence. Whereas previously, the use of fentanyl and fentanyl derivatives was the main point of attention, legislations have reacted accordingly, which may have been a driving force towards the (ab)use of alternative µ-opioid receptor (MOR) agonists. In contrast to fentanyl (analogues), details on these novel non-fentanyl opioid NPS are scarce. However, the increasing amount of case reports on (sometimes fatal) intoxications involving these drugs stresses the danger of this knowledge gap. Methods: We investigated the biological activity of a panel of 11 ‘alternative’, newly emerging MOR agonists (2-methyl-AP-237, AP-237, bromadol, brophine, butorphanol, isotonitazene, mitragynine, 7-OH-mitragynine, MT-45, piperidylthiambutene and tianeptine) using two previously reported, closely related in vitro MOR activation assays monitoring either G protein (mini-Gi) or β-arrestin2 (βarr2) recruitment. The assays are based on the functional complementation of a split nanoluminase (NanoLue Binary Technology®, Promega): activation of MOR, fused to one part of the nanoluminase, leads to recruitment of either βarr2 or mini-Gi, fused to the other part. This results in a restoration of the enzymatic activity, producing a measurable bioluminescent signal upon addition of a substrate. This set-up also allowed the assessment of biased agonism via previously published equations with hydromorphone as an unbiased reference compound. Results: Activity profiles were obtained for all tested compounds, with values for potency (EC50) ranging from 1.89 nM (bromadol) to >3 µM (AP-237 and tianeptine). Bromadol, brophine, isotonitazene, piperidylthiambutene and tianeptine had the highest efficacy (Emax) values, exceeding that of the reference compound hydromorphone ≥1.3-fold (βarr2 assay) and >2.6-fold (mini-Gi assay). In both assays, butorphanol, mitragynine and 7-OH-mitragynine were partial agonists compared to hydromorphone, the reference compound. No statistically significant bias was found for any of the tested MOR agonists. Conclusions: This study is the first to systematically investigate the in vitro biological activity of a diverse panel of emerging non-fentanyl opioid NPS at MOR. Pharmacological profiling
of such novel substances is crucial to make a realistic estimation of the potential danger their use might bring along. This, in turn, may allow to prioritize legislative efforts towards controlling (variants of) these emerging drugs. Given the high potencies and efficacies of many compounds in the studied panel, intensive monitoring and proactive control measures remain of paramount importance.

First report on brorphine: the next opioid on the deadly new psychoactive substances’ horizon?
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Background: New psychoactive substances (NPS) continue to appear on the drug market. Until recently, new synthetic opioids, which are amongst the most dangerous NPS, primarily encompassed analogues of the potent analgesic fentanyl. Lately, also other new synthetic opioids have increasingly started to surface. This is the first report on the identification and full chemical characterization of online sourced brorphine, a novel potent synthetic opioid with a piperidine benzimidazolone structure. Brorphine was identified in a powder and quantified in two serum samples of a patient seeking medical help for detoxification. Methods: Chemical characterization of the powder was performed by liquid chromatography high resolution mass spectrometry (LC-HRMS), gas chromatography (GC)-MS, LC diode array detection (DAD), Fourier-transform infrared (FT-IR) and NMR spectroscopy analyses. In vitro biological activity of brorphine was determined by a cell-based µ-opioid receptor (MOR) activation assay. Patient samples were analyzed with a quantitative LC-HRMS method and with the bio-assay. Results: LC-HRMS identified an exact mass of m/z 400.1020 and 402.1005 for the compound, corresponding to both bromine isotopes. GC-MS, LC-DAD and FT-IR spectra were obtained from the powder. 1H- and 13C-NMR-analyses confirmed the structural configuration of brorphine. An EC50 of 30.9 nM (13.5 ng/mL) and an Emax of 209%, relative to hydromorphone, were derived from the bio-assay, confirming the high potency and efficacy of this compound. The potency of brorphine approaches that of the potent analgesic fentanyl (EC50 of 18.7 nM). In two serum samples of the patient (taken approximately 60 hours apart), brorphine and a hydroxyl metabolite were found using the LC-HRMS method. The obtained brorphine concentrations in the first and second serum sample were 69.4 and 7.9 ng/mL, respectively. The presence of opioid activity in the serum was also confirmed via the MOR activation assay. Conclusions: The occurrence of brorphine is yet another example of how the illicit drug market is continuously evolving in an attempt to escape international legislation. The online availability of this compound, combined with its non-scheduled nature in many countries, its unequivocal identification in serum samples from a patient, and the demonstration that it acts as a strong MOR agonist: all these aspects should alert toxicology labs, as new cases - including fatalities involving brorphine - may emerge.

Spice stories from the slammer - real life experiences of NPS users in prison
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NPS have posed significant difficulties in UK prisons over the last 5 years. Specifically, synthetic cannabinoids have become the most widely used drug in UK prisons, contributing directly to instability, disruption, debt and prison staff and prisoners feeling less safe. Part of the challenge is fully understanding the reasons people use 'Spice' and the impact that it has on them - only then can we provide the support that will be effective. At HMP & YOI Parc we have embarked on an ambitious educational project, capturing real life 'Spice stories' from the residents that reside with us. We hope that these perspectives will help us all to better understand the many issues associated with the use of NPS so that we can shape our solutions to suit.