The IACFT Gazette

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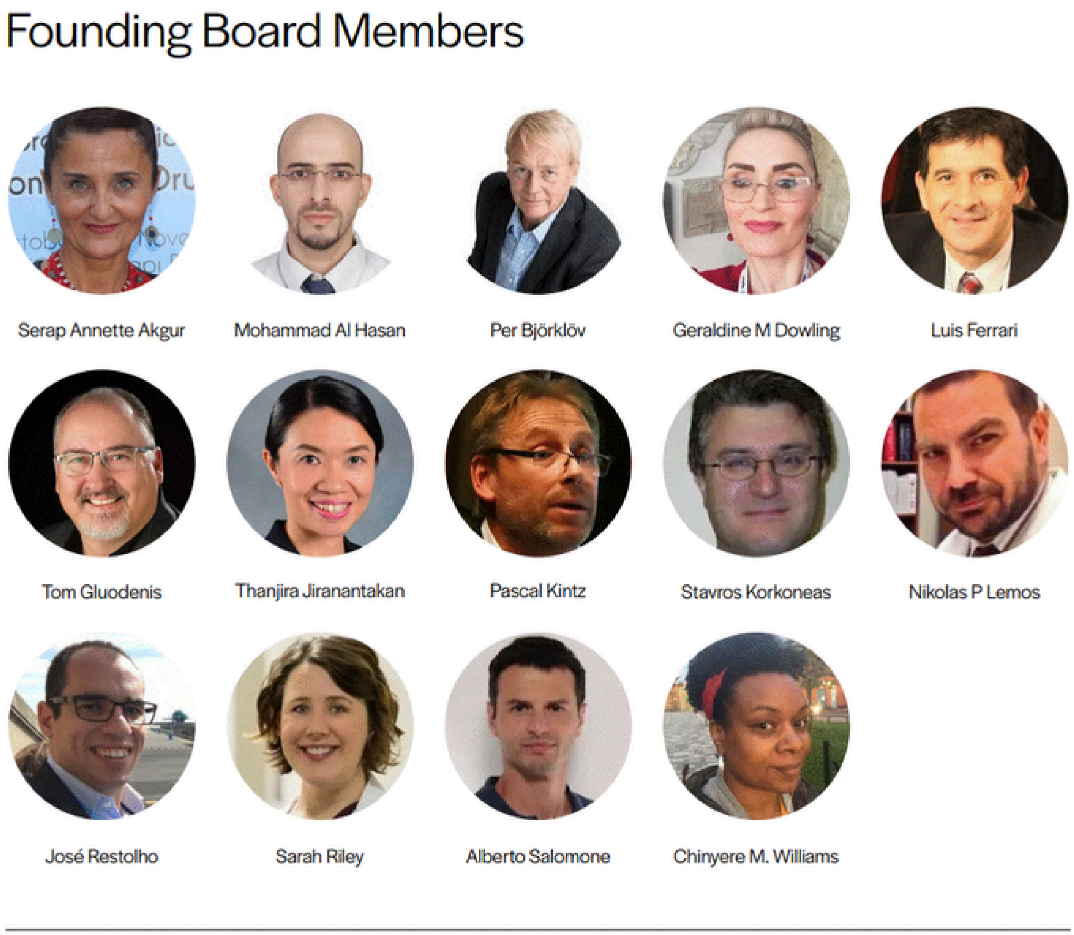
IACFT Gazette Editor in Chief

Dr Geraldine M. Dowling SFHEA



If you are interested in serving on —Young Scientist, Communications or Toxicology Certification Continuing Education committees—please contact the Editor-in-Chief

Welcome

There is much to celebrate at the International Alliance of Clinical and Forensic Toxicologists (IACFT) and we are both delighted and honored to present the second issue of the IACFT Gazette and launch its new website. This professional magazine features peer-reviewed articles and other content curated by our editorial team, undergraduate and postgraduate students to inform and engage our readership. Adding to our excitement, we are thrilled to announce that we will be publishing more articles, including case reports, brief preliminary research reports, mini reviews, data reports and original research papers. These will cover topics within IACFT disciplines but also peer reviewed knowledge from outside IACFT fields in the "Science in Motion" section thus offering an even richer experience for our readership in the future.

IACFT is a global consortium of scientists dedicated to serving their local communities through rigorous research and application of forensic and clinical toxicology principles. Despite the challenges of time and budget constraints that often prevent members from attending professional meetings in distant locations, IACFT remains committed to fostering productive relationships with students and professionals utilising tools from Community Based Learning (CBL) and Universal Design for Learning (UDL) pedagogical approaches thereby making meaningful contributions to the field of toxicology. Our efforts are grounded in comprehensive empirical studies and high-quality data.

We are confident that past attendees of online IACFT meetings and readers of the IACFT Gazette have appreciated the invaluable insights gained from both the scientific presentations and the dynamic discussions that ensued. In addition, the professional magazine and other online education resources will all be offered for free. We extend our commendation to previous meeting organizers, speakers, sponsors and editors for their exceptional efforts in conducting and editing past events seamlessly, before, during and since the pandemic. The availability of these meetings in multiple languages (by request) had significantly contributed to accessibility and attracting virtual participation from 65 countries worldwide at online meetings.

In recognition of our achievements and the vibrant community we serve, we have decided to launch a new website to host the IACFT Gazette magazine and information related to toxicology. This website and publication is aimed at professionals, academics,

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researchers and students within clinical and forensic toxicology disciplines but also readership outside of those fields. The partners of IACFT, including those in industry and higher education represent diverse communities. Therefore, inclusion remains a high priority on the IACFT Gazette's agenda. It is essential that we provide a welcoming environment for all, where everyone feels valued, respected and appreciated. As we move forward together, we are committed to fostering inclusion and celebrating diversity in all aspects of forensic and clinical toxicology research, education and service provision.

The IACFT Gazette is published exclusively in digital format and is shared online. Consequently, our publications may incorporate a variety of media elements, including audio and visual files, various image formats and interactive hyperlinks to websites and other online resources.

Our new IACFT Gazette website is accessible by pasting the following into a browser:
https://theinternationalallianceofclin.godaddysites.com/

Archived on demand selected meetings are available by pasting the following into a browser:
https://forensiceducation.brightspace.com/d2l/hom

These past events were free of charge and selected content was considered suitable for submission for continuing education credits.

We hope you will enjoy the next issue of the IACFT Gazette and its new website. Please feel free to provide feedback or suggestions. We are particularly interested to receive 1.5 page case report articles for publication in future issues of the IACFT Gazette.

Happy reading!
Geraldine

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Examples:
Reference to a journal publication:
[1] Meneton P, Jeunemaitre X, de Wardener HE, et al. Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. Physiol Rev. 2005;85:679–715.

Reference to a book:
[2] Wenger NK, Sivarajan Froelicher E, Smith LK, et al. Cardiac rehabilitation. Rockville (MD): Agency for Health Care Policy and Research (US); 1995.

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Academic Partners

There is much to celebrate at the International Alliance of Clinical and Forensic Toxicologists (IACFT), and we are both delighted and honored to present the second issue of the IACFT Gazette and the launch of the new IACFT Gazette website.

We would like to acknowledge grant funding for supporting this endeavor from the Atlantic Technological University (formerly Institute of Technology Sligo), Teaching and Learning Centre, Sligo, Ireland. This funding was approved through the National Forum for Teaching and Learning's Strategic Alignment for Teaching and Learning Enhancement Fund 2022. In addition, funding was obtained from the National Technological University Transformation for Recovery and Resilience (N-TUTORR) program 2023/24 which originated from the European Union and the Higher Education Authority in Ireland.

The support secured was utilised to help undergraduate students, IACFT professionals, academics, researchers, postdoctoral fellows and postgraduate students from various disciplines share or create learning resources for our readership and to publish interesting, exciting, unusual or otherwise noteworthy peer reviewed material in the IACFT Gazette. In addition to produce alternative learning resources using Universal Design for Learning (UDL) and Community Based Learning (CBL) pedagogies that facilitate the sharing of information in multiple formats with students, professionals and the general public thus making IACFT accessible to those with non-traditional learning behaviours and to everyone irrespective of toxicology knowledge or career stage.

We welcome feedback on improving our accessibility and inclusivity for all contributors and readers.

IACFT wishes to acknowledge our academic and educational partners who help us bring virtual knowledge to every part of the world.

An attempted suicide defense case study involving alprazolam and driving

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Keywords: Xanax, Alprazolam, Driving Under the Influence of Drugs, Impaired Driving

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Alprazolam (Xanax®), used to treat anxiety disorders and panic attacks, is the most frequently prescribed benzodiazepine in the United States. Alprazolam misuse and abuse are common, either alone or in combination with alcohol. In Alabama, alprazolam was the number one drug found in driving cases in 2014. In 2023, alprazolam was the fifth most detected drug and 95% of drivers were Caucasian with a median age of 35 years old. The typical therapeutic range for alprazolam has been cited as 10-100 ng/mL. However, DUID cases routinely exceed this concentration range. The crash risk associated with the abuse of benzodiazepines such as alprazolam is 2-10 fold.

Blood specimens were screened by EIA using a Randox Evidence Analyzer Plus and the Ultra biochip assay. Quantitation was performed by liquid-liquid extraction followed by analysis with an Agilent 6460 Triple Quadrupole LC/MS/MS. Ethanol was screened by HS/GC/MS and quantitated by an HS/GC dual capillary flame ionization detector.

Case Study: A 53-year-old Caucasian male drove off the roadway into a residential house, killing an elderly disabled woman in her sleep. The subject was disoriented and extremely combative, refusing to cooperate with officers and medical personnel. Law enforcement ultimately tased the subject in order to remove him from the vehicle. Toxicological analysis revealed the following findings:

Table 1: Blood concentrations in case study.

Target	Blood concentrations
Ethanol	0.129%
Benzoylegonine	160 ng/mL
Alprazolam	1400 ng/mL
Clonazepam	<10 ng/mL

During courtroom testimony, the defendant admitted to taking 188 mg of alprazolam (i.e., 160 x 1 mg + 14 x 2 mg pills), 80 mg of clonazepam (i.e., 40 pills), and several shots of vodka. He stated that his intention was to commit suicide and he did not have recollection of driving the evening of the crash. For comparison, the equation "Dose = Concentration x Volume of Distribution (Vd)" was used to estimate the theoretical concentration achieved from 188 mg of alprazolam, with Vd = 1.3 L/kg, in a 170 lb male. The estimated blood concentration was calculated to be 1,880 ng/mL.

Alprazolam at 1,400 ng/mL remains the highest concentration ever recorded in a driving case at ADFS. This is the first known "attempted suicide" defense challenge in a DUID case. Furthermore, over 50% of laboratories in the United States have stop testing limits for ethanol where drug testing is not conducted if an ethanol threshold is met (e.g., 0.08 or 0.10%). In such cases, the contribution of alprazolam would have been missed

Acronyms:

Driving Under the Influence of Drugs (DUID), Enzyme Immunoassay (EIA), Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS), Alabama Department of Forensic Sciences (ADFS), Headspace Gas Chromatography Mass Spectrometry (HS/GC/MS), Headspace Gas Chromatography (HS/GC)

References:

Leufkens T, Vermeeran A, Smink BE, van Ruitenbeek P, Ramaekers JG. Cognitive, Psychomotor and Actual Driving Performance in Healthy Volunteers after Immediate and Extended Release Formulations of Alprazolam 1 mg. Psychopharmacology 2007;191:951–9.

Opiate positivity due to opium poppy consumption

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Keywords: Opiate Positivity, Poppy Seed, Workplace Drug Testing, Forensic Toxicology, Poppy Seed Defense, Gas Chromatography Mass Spectrometry, Liquid Chromatography Mass Spectrometry

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Introduction:

Since ancient times, the poppy plant, *Papaver somniferum L.*, has been used for different purposes; for medicinal purposes, as a foodstuff for nutritional value and also illegally for euphoric effects. The latex of the opium poppy *Papaver somniferum L.* contains pharmaceutically important alkaloids such as morphine, codeine, thebaine, papaverine and noscapine [1]. Latex is harvested by growers who cut the pods before they ripen. Latex flows out through these cuts, and when dried, it is collected as raw opium [2]. Opium alkaloids are not normally present in poppy seeds, but they may be contaminated with latex during harvest. Harvesting method and geographical origin may affect the content of morphine, codeine, and thebaine in the husk of poppy seeds [3].

In addition, unwashed and untreated poppy seed pods may have higher alkaloid contents than washed poppy seed pods. Some opium alkaloids, including morphine, codeine and thebaine are narcotic drugs under international control [4]. The European Food Safety Authority (EFSA) published a report on alkaloids in poppy seed food products and conducted a public health risk assessment. In the report, it was hypothesised that servings of foodstuffs with high poppy seed content could result in morphine exposure in the range of 38-200 μg/kg body weight per serving for adults [5].

There is no common legislation, and the situation in each country is different. In some countries, such as China and other Asian countries, the use of poppy seeds for food is prohibited [6]. In Belgium, the use of poppy seeds is prohibited in all foods except bakery products [5]. To avoid these problems, low-morphine seed varieties are certified for cultivation in Germany, ('Przemko') and Austria ('Edel-Weiß', 'Edel-Rot', 'Florian', 'Josef', 'Zero' and 'Zero 2000'). While there are cases of opiate positivity in biological materials when poppy seeds are consumed even in small doses, there is no legislation in Europe setting maximum limits for alkaloids in poppy seeds or poppy foods. The Substance Abuse and Mental Health Service (SAMHSA) regulated the opiate cut-off value from 300 ng/mL to 2000 ng/mL for clinical cases only in 2010. In Türkiye, similar legislation was passed in 2016 and the cut-off value was increased to 2000 ng/mL not only for clinical but also for Workplace Drug Testing (WDT) cases.
Türkiye has a longer tradition of poppy seed consumption than other countries and is the world's largest legal producer of poppy. The use of poppy seed products is widespread in Türkiye and more than 100 g of poppy seeds can be used in one meal [7]. There are studies in the literature on the effect of poppy seed consumption on opiate positivity in the WDT screening test and forensic case results [7–11]. ElSohly et al. (1989) suggested that urine codeine concentrations >300 ng/mL and morphine-to-codeine ratios <2 were indicative of codeine intake and ruled out poppy seed consumption for test results [12]. However, in a few studies, codeine predominance was detected in poppy seed samples [3,13–15].

WDT differ between countries. Companies implementing WDT conduct drug tests on urine samples from all job applicants before recruitment, randomly from current employees, after an occupational accident or for a comprehensive assessment in suspicious cases and evaluate all results. The urine guidelines produced by the European Workplace Drug Testing Association (EWDTS), first published in 2002 and updated in 2015, are structured to establish optimal procedures while allowing each country to operate within its regional legislative requirements [16]. In Türkiye, some employers want to test their employees for drugs and alcohol to monitor their work activities and to implement occupational health and safety requirements. They apply to the relevant institutions and have their employees tested for drugs and alcohol periodically or at indefinite intervals. Since there is no special regulation regarding workplace substance testing in Türkiye, these tests are carried out in accordance with the instructions of the Ministry of Health. /12/2016 [17]. There are many convenient immunological and chromatographic analytical techniques for the detection and quantification of opium alkaloids in urine. In analyses performed for non-clinical forensic purposes, it is essential to analyse the sample with two different methods. The immunoassay method, which is among the techniques recommended for urine substance screening based on the EWDTS urine guidelines and is usually applied as the first step for urine substance screening because it is fast and inexpensive. In the case of positive screening results, the drugs should be confirmed using a chromatographic technique with mass spectrometry (e.g. GC-MS or LC-MS/MS). In WDT, the participants are informed about the compounds detected in the confirmatory tests. In this study, the verification results of 5 cases that were found to be opiate-positive in the urine screening test collected from people who were tested for drugs in the workplace but who had no previous history of drug use and the information obtained from the interviews were evaluated together.

Figure 1: Poppy seed pastry (authors own photo)

Material and Methods:

Urine samples were analysed by enzymatic immunoassay method (OLYMPUS AU400) opiates, and creatinine-detect assay immunoassay reagents were purchased from Thermo-Fisher Scientific (Massachusetts, USA). Analyses were performed on each sample according to the manufacturer's recommended instructions: Cut-off concentration for opiates used 300 ng/mL, and the normal ranges for creatinine were 20–300 mg/dL. Positive urine screening test results in opiates were confirmed using validated gas chromatography mass spectrometry (GC–MS) and Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) methods.

Case Series :

Case 1: A 23-year-old male administered urine WDT at the time of job application. He stated that he had consumed a poppy seed muffin the night before the test. The screening analysis detected opiates (366 ng/mL). Morphine, neopine and codeine were found to be positive in the confirmatory analyses performed using GC–MS and LC-MS/MS.

Case 2: A 27-year-old man tested positive for opiates (5607 ng/mL) in a random WDT test. He declared that he had smoked mixed hookah (unknown ingredients), consumed herbal tea, and reported consuming a very spicy, highly alcohol of a famous brand two days before the test. Confirmatory analyses results were positive for morphine, codeine and neopine.

Case 3: A 20-year-old man was found to be positive for opiates (>300 ng/mL) in random WDT tests and reported having consumed cake with poppy seeds the night before the test. In confirmatory analysis, morphine, thebaine, codeine and erucilamide were found to be positive.

Case 4: A 30-year-old man was found to be positive for opiates (1198 ng/mL) during screening. He declared that he had consumed chips with poppy seeds three days earlier. Codeine, neopine and thebaine were found positive in GC–MS analysis.

Case 5: A 27-year-old man was found to be positive for opiates (2392 ng/mL) in random WDT tests. He took painkillers prescribed by a physician for leg pain 2-3 weeks ago before the test and stated that he had eaten homemade poppy paste 12 h ago. Codeine, neopine and thebaine were found to be positive in the confirmatory analysis.

Results and Discussion:

In all cases, confirmatory analyses were positive for neopine, thebaine, papaverine and codeine alkaloids derived from poppy seeds in addition to morphine.

Poppy seed cake, muffins, and paste (Cases 1-3-5) contain high concentrations of poppy seeds [3]. There is no information on whether the poppy seeds in the final food product were cooked or not. As reported by Case 2, the herbal ingredient present in the highly spicy and alcoholic drink, which he considered to be the cause of opiate positivity, was stated on the label. However, the drink product was analysed and toxicological analysis was negative. Case 4 claims to have had a positive result after consuming poppy seed crisps. In many studies, alkaloids such as papaverine and thebaine have been analysed in urine samples from people who consumed poppy seeds and inconsistencies have been reported in their occurrence in different seed varieties and in urine following poppy seed ingestion [18,19]. Studies on opium alkaloids are mostly related to morphine, and other alkaloids, especially noscapine, narcein, and oripavine have been less well studied. Therefore, further studies on alkaloids present in poppy-containing food products are needed to assess consumer exposure and risk assessment of their consumption [14]. Carlin et al. reported that morphine, codeine, thebaine and noscapine alkaloids were analysed in poppy seeds from eight countries, including Türkiye, Türkiye was the lowest country in terms of morphine and codeine concentrations, where thebaine and noscapine were not found at all [3]. In the review presented by Lachenmeier et al., it was pointed out that losses during food processing were not controlled in most studies that gave opiate positivity resulting from poppy seed consumption and that washing of morphine seeds and other pre-treatments can reduce morphine levels by up to 90% after cooking, roasting, grinding and there is a large decrease in morphine content. Therefore, he emphasised that the urine results were misinterpreted. In addition, he stated that poppy seeds used in the past are no longer used today, producers use certain commercial products, and it is not possible to exceed the cut-off limit for tests [20]. In countries such as Türkiye, where poppy seeds are widely used as food products, the analysis and evaluation of alkaloids of food origin within the scope of workplace drug testing, as well as information and regulations on this subject are very important. Although our study has shown that the toxicological interpretation of the intentional or unintentional intake of poppy seed foods depends on food intake, this may represent a trap. Therefore, it is important to keep in mind that there are question marks regarding the use of the “poppy seed defence”.

Acronymns:

Work Place Drug Testing (WDT), Gas Chromatograph Mass Spectrometry (GCMS), Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS), SAMSHA (Substance Abuse and Mental Health Service), EWDTS (European Workplace Drug Testing Association)

References:

[1] Moreno P, R. Van Der Heijden, Verpoorte,R. Effect of terpenoid precursor feeding and elicitation on formation of indole alkaloids in cell suspension cultures of Catharanthus roseus, PlantCell Reports, 1993 Oct;12(12):702-5.

[2] Powers D, Erickson S, Swortwood MJ, Quantification of Morphine, Codeine, and Thebaine in Home-Brewed Poppy Seed Tea by LC-MS/MS, J. Forensic Sci. 2018;63 :1229–1235.

[3] Carlin MG, Dean JR, Ames JM, Opium Alkaloids in Harvested and Thermally Processed Poppy Seeds, Front. Chem. 2020;8:737.

[4] UNODC, Annual Estimates Of Requirements For Narcotic Drugs, Manufacture Of Synthetic Drugs , Cultivation Of The Opium Poppy, 2023. https://www.unodc.org/documents/crop monitoring/Afghanistan/Afghanistan_opium_survey_2023.pdf Accessed 27/07/24

[5] EFSA, Scientific Opinion on the risks for public health related to the presence of opium alkaloids in poppy seeds. 9 (2011) 1–150. https://doi.org/10.2903/j.efsa.2011.2405. Accessed 27/07/24

[6] Go Q, Zhang J, Zhao S, Sham B, Determination of Five Alkaloids of Pericarpium Papaveris in Hot Pot Broth Using Ultra-Performance Liquid Chromatography Coupled to Triple Quadruple Mass Spectrometry, Food Anal. Methods. 2013;6:698–704.

[7] Özbunar E, Aydoğdu M, Döğer R, Bostanci,HI, Koruyucu M, Akgür, SA Morphine Concentrations in Human Urine Following Poppy Seed Paste Consumption, Forensic Sci. Int. 2019;295:121–127.

[8] Narcessian EJ, Yoon HJ. False-positive urine drug screen: beware the poppy seed bagel. Pain Symptom Manage. 1997;14: 261–263.

[9] Rohrig TP, Moore C. The determination of morphine in urine and oral fluid following ingestion of poppy seeds, J. Anal. Toxicol. 2003:27:449–452.

[10] Smith ML, Nichols DC, Underwood P, Fuller Z, Moser MA, LoDico C, Gorelick DA, Newmeyer M, Concheiro M, Huestis MA. Morphine and codeine concentrations in human urine following controlled poppy seeds administration of known opiate content. Forensic Sci. Int. 2014;241:87–90.

[11] Samano KL, Clouette RE, Rowland BJ, Barry RJ. Sample Concentrations of Morphine and Codeine in Paired Oral Fluid and Urine Specimens Following Ingestion of a Poppy Seed Roll and Raw Poppy Seeds, J. Anal. Toxicol. 2015;39: 655–661.

[12] ElSohly MA, Jones AB, Morphine and Codeine in Biological Fluids: Approaches to Source Differentiation., Forensic Sci. 1989;1: 13–22.

[13] Avula B,Katragunta K, Adams SJ, Wang YH, Chittiboyina AG, Khan IA, Applicability of LC-QToF and Microscopical Tools in Combating the Sophisticated, Economically Motivated Adulteration of Poppy Seeds, Foods. 2023;12: 1510.

[14] Casado-Hidalgo G, Martínez-García G, Morante-Zarcero S, Pérez-Quintanilla D, Sierra I New Validated Method for Determining Six Opium Alkaloids in Poppy Seed-Containing Bakery Products by High-Performance Liquid Chromatography-Tandem Mass Spectrometry after Magnetic Solid-Phase Extraction, J. Agric. Food Chem. 2022;70:7594–7606.

[15] López P, Pereboom-de Fauw DPKH, Mulder, PPJ, Spanjer M, de Stoppelaar J, Mol HGJ, de Nijs M. Straightforward analytical method to determine opium alkaloids in poppy seeds and bakery products, Food Chem. 2018;242: 443–450.

16] EWDTS. European Guidelines for Workplace Drug Testing in Urine 2015-11-01, (2015) 1–40. http://www.ewdts.org/data/uploads/documents/ewdts-urine-guideline-2015-11-01-v2.0.pdf. Accessed 27/07/24

[17] Sağlık Bakanlığı, İdrar Numunelerinde Yasadışı ve Kötüye Kullanılan İlaç ve Madde Analizi Yapan Tıbbi Laboratuvarlar ile Madde Bağımlılığı Teşhis ve Tedavi Merkezlerindeki Tıbbi Laboratuvarların İşleyiş Esasları, (2016). http://dosyamerkez.saglik.gov.tr/Eklenti/2533,genelge201422maddebagimliligipdf.pdf, Accessed 27/07/24

16] EWDTS. European Guidelines for Workplace Drug Testing in Urine 2015-11-01, (2015) 1–40. http://www.ewdts.org/data/uploads/documents/ewdts-urine-guideline-2015-11-01-v2.0.pdf. Accessed 27/07/24

[17] Sağlık Bakanlığı, İdrar Numunelerinde Yasadışı ve Kötüye Kullanılan İlaç ve Madde Analizi Yapan Tıbbi Laboratuvarlar ile Madde Bağımlılığı Teşhis ve Tedavi Merkezlerindeki Tıbbi Laboratuvarların İşleyiş Esasları, (2016). http://dosyamerkez.saglik.gov.tr/Eklenti/2533,genelge201422maddebagimliligipdf.pdf. Accessed 27/07/24

[18] Paul BD, Deka C, Knight ES, Smith ML, Gas chromatographic/mass spectrometric detection of narcotine, papaverine, and thebaine in seeds of Papaver somniferum., Planta Med. 1996;62: 544–547.

[19] Trafkowski J, Madea B, Musshoff F, The significance of putative urinary markers of illicit heroin use after consumption of poppy seed products., Ther. Drug Monit. 2006:28:552–558.

[20] Lachenmeier DW, Sproll C, Musshoff F. Poppy Seed Foods and Opiate Drug Testing-Where Are We Today?, Ther. Drug Monit. 2010;32:11–18.

Diethylene glycol (DEG): clinical and forensic remarks in victims of massive intoxication. An update

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Keywords: Diethylene Glycol, Massive Intoxication, Biotransformation, Postmortem Analysis, Clinical Features, Lethal Dose

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Abstract:
Among the toxic glycols, diethylene glycol (DEG) is the one that has caused the most episodes of mass poisonings around the world in the last century [1]. In addition to its high toxicity [2]. It has histopathological consequences in various organs and in the internal milieu, which justifies the continuous monitoring of scientific advances. The biotransformation of DEG has not been fully understood. Half a century ago it was believed that DEG broke down in the human body generating ethylene glycol and further deposition of calcium oxalate crystals causing severe tubular damage [1]. Wiener and Richardson's experiments on mice in 1989 [3] showed that the DEG molecule did not break down but formed HEAA (ethoxy hydroxy acetic acid) that produces severe tubular necrosis but not renal crystal deposition. This circumstance could be verified in an Argentinian case. In this short review we provide some current data in Argentina with some episodes reported in different clinical and forensic aspects considering mass poisonings reported in the literature,

Introduction:
For nearly a century, several mass poisonings with DEG have occurred around the world. Most of these episodes have occurred in developing countries and have been caused by contamination of pharmaceutical preparations, whose excipient solvents have been replaced by DEG [1,4-6]. In many of these episodes the victims were young children [4-5]. However, surprisingly, in several mass poisoning episodes, DEG was in high concentrations (>40%) [1,7], while in other poisonings the victims were adults, in which pharmaceutical preparations contained low DEG (<30%) when the cases were analyzed retrospectively and through medical records. In poisoning episodes with high concentrations of DEG, clinical symptoms and laboratory data are usually critical. For example, in our Argentinian case, all those who took drugs with excipients contaminated with DEG (50-60%), the symptoms appeared a few hours after consumption and patients had to be hospitalized with marked anuria, hepatopathy with high values of the enzymes GOT (glutamic oxaloacetic transaminase), GPT (glutamic pyruvic transaminase) and metabolic acidosis [1] In 1992 in Argentina, an episode of mass deaths from propolis syrups in which propylene glycol was replaced by DEG occurred. Twenty-nine people died and hundreds had to be hospitalized. In our judicial laboratory, more than 20 postmortem cases were set aside, 15 of them to study including anatomopathological, analytical postmortem and clinical aspects. This large intoxication allowed a series of conclusions to be achieved.

Some episodes reported in the literature:
As can be seen in Table I, in almost all cases, the source of poisoning was the excipient or liquid vehicle of pharmaceutical preparations. This demonstrates a failure in the control of excipients, especially the solvents commonly used to dissolve the drugs (mainly propylene glycol and/or glycerine). It is striking that, despite so many episodes with dozens of cases, these fatal poisonings continue to recur. Some episodes were triggered in a short period of time and on a massive scale (e.g. the case of Argentina and

the United States among others) with a percentage of toxic excipients exceeding 50-60% [1,7], while others emerged from an epidemiological analysis over a period of weeks or months (e.g. the recent case in Gambia, Panama, Haiti) [4,8-9].

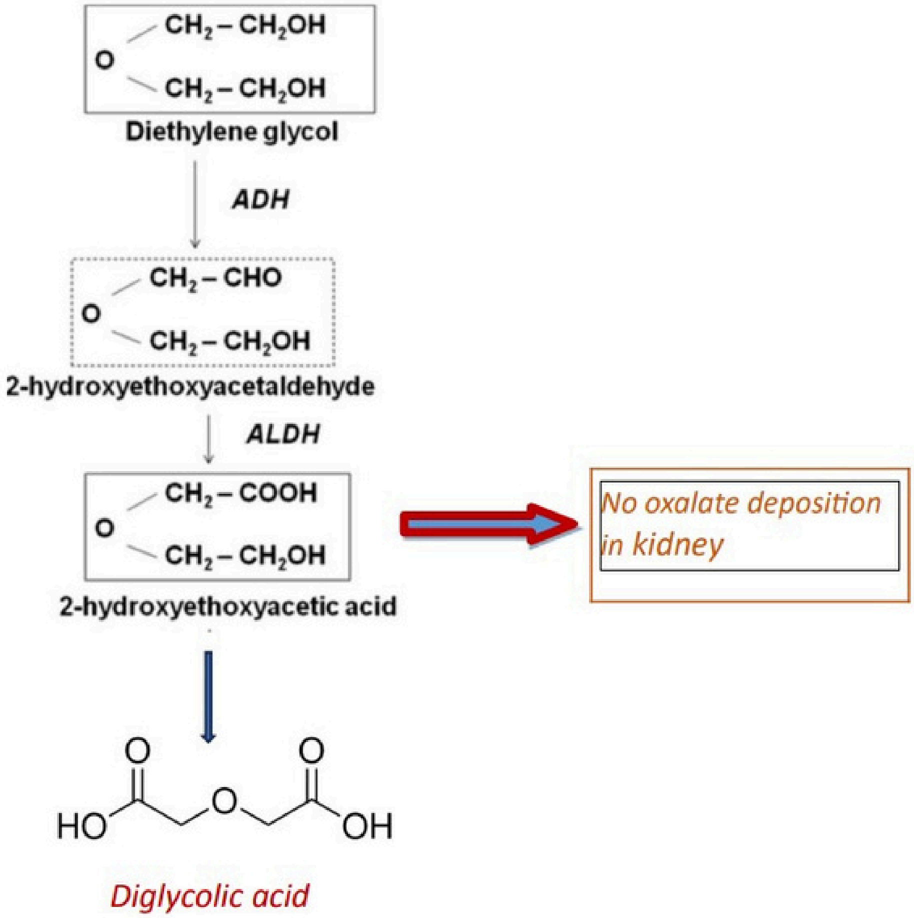
Table 1: Some mass poisoning episodes reported in the literature [1-9]

Country	year	Fatalities	Contamination source
USA	1937	105	Excipient
South Africa	1967	7	unknown
Spain	1985	5	Excipient
India	1986	14	Excipient
Nigeria	1990	47	Excipient
Bangladesh	1990	51	Excipient
Argentina	1992	29	Excipient
Haiti	1996	89	Excipient
Panama	2006	365	Excipient
Brazil	2019	4	Food contamination
Gambia	2022	67	Excipient (child < 2y)

Biotransformation and histopathological observations:

In Winek et al. experiments, an increase was observed in the level of oxalate excreted in the urine of male rats [10] This indicates that the ether bridge can, in principle, be split; however, the oxalic acid concentrations in the blood and kidneys after administration of diethylene glycol remain lower than after administration of the same amounts of ethylene glycol. After a single oral or intravenous dose of 14C-labelled diethylene glycol of 1.1 g/Kg body weight, no ether cleavage products were found in the urine of male rats, only the administered substance and after 6 and 12 hours about 20 % and 32 % of the dose was recovered as HEAA. Contamination with monoethylene glycol has been suggested in other studies as the source of oxalic acid. After inhibition of alcohol dehydrogenase (ADH) with pyrazole the authors found almost exclusively diethylene glycol in urine and no HEAA. The acute toxicity was also lowered by pyrazole, which indicates that the metabolites are the cause of the nephrotoxic effects according to Wiener and Richardson experiments [3]. The latest authors stated that the main metabolite found was HEA (Fig. 1)

Figure 1: DEG pathway biotransformation (modify. [11])



However, recently, de Almeida Araújo and co-workers [12] consigned that diglycolic acid (DGA), formed from HEAA, is related to kidney injury. DGA is filtered in the glomerulus and transported to the proximal tubule. There, DGA inhibits the enzyme succinate dehydrogenase, which causes cell death by blocking the production of adenosine triphosphate. Therefore, the substance acts by interfering with both energy mitochondrial oxygen production and use.

In our Argentinian case [1], postmortem histopathological examinations of the kidneys of the victims showed intense tubular cortical damage, limited to the proximal convoluted tubules, with cellular ghosts and cells in pyknosis or karyorrhexis. No crystal deposition was observed in the tubule walls, suggesting that in humans, DEG follows a different metabolic pathway than was hypothesized decades ago. The breakdown of the oxygen bridge of the ether function would not occur and the tubular damage would come from the direct action of HEAA and secondarily DGA, thus explaining the anuria, in addition to the metabolic acidosis and liver damage,observed in all deceased intoxicated individuals.

The liver showed hepatocytic stellate colliquative necrosis affecting a large majority of hepatic lobules with neutrophilic leukocyte infiltration and marked global vascular congestion.
In the South African poisoning in 1967 [13], post-mortem histopathological examination showed tubular necrosis and centrilobular hydropic degeneration of the liver. The first toxic epidemic in the USA and in India gave the same histopathological result [7,14].

Clinical findings:
In our Argentinian massive intoxication, the medical records of all fatalities showed an increase in serum levels of liver enzymes, such as GPT, GOT, GGT (gamma glutamyl transpeptidase) and LDH (lactate dehydrogenase), all from medium to severe levels (>50-200 UI/L; >34-150 UI/L; >30 UI/l; >220 UI/l, respectively). Fifteen cases showed significant levels of creatine phosphokinase (CK) and progressive increase of leukocytes (up to 17,000 /mm3). Hematocrit decreased in all cases. Urea and creatinine increased until haemodialysis and/or plasmapheresis. Oliguria manifested within a short period of time (2 to 12 hours after ingestion) leading to complete anuria within 24 to 48 hours. All cases showed metabolic acidosis. This was one of the salient clinical features of the case and contrasts with the opinion of some authors that metabolic acidosis is moderate or mild [1].

In our cases all were medium to severe with the latter condition prevailing (see Table 2). In 12 cases the body temperature rose to 38 or 39°C once the characteristic symptom picture began. Four victims showed QT segment elongation on the electrocardiogram.

Table 2. Acid-base parameters in the 15 cases studied in Argentinian episode.

Victim	Gender	Age	pH	Base excess (BE) (mEq/L)	Anion Gap (mEq/L)
1	M	70	7.31	-13.1	34.4
2	M	59	7.21	-12.0	32.0
3	F	57	7.21	-11.1	33.1
4	M	60	7.42	-12.5	29.0
5	F	93	7.32	-11.5	30.5
6	M	62	7.36	-12.9	31.0
7	F	54	7.21	-9.1	31.7
8	F	60	7.24	-8.7	30.0
9	M	66	7.32	-8.9	31.5
10	M	50	7.45	-8.5	29.8
11	F	56	7.1	-8.0	28.6
12	M	83	7.3	-8.0	27.8
13	F	59	7.36	-8.8	28.0
14	F	65	NR	NR	NR
15	F	66	NR	NR	NR

DEG lethal dose calculations in the Argentinian episode:

We describe in this review in detail how the lethal dose was calculated for the Argentinian episode. The DEG values in g/Kg body weight were obtained from the ratio between the incorporated DEG (5 mL to 20 mL), considering the DEG concentration in the propolis syrup bottles, belonging to each victim and provided by the relatives. Also, the victim's body weight was taken from the medical history and autopsy report.

From the data below, for each victim, the percentage of DEG (w/v) in propolis syrup and the respective figure for minimum intake of syrup (5 mL) and maximum (20 mL) of syrups contaminated with DEG, and in the percentage were indicated in column 3 of Table 3. The density unit of DEG: 1,118 g/mL was used.

Table 3: DEG (% w/v) and PG (% w/v) identified in samples of propolis syrup and lethal dose.

			DEG (g) if	DEG (g) if	DEG g/Kg bodyweight	DEG mL/Kg bodyweight	DEG g/Kg bodyweight	DEG mL/Kg bodyweight
Victim	%DEG	%PG	consumed 5 mL	consumed 20 mL	if consumed 5 mL	if consumed 5 mL	if consumed 20 mL	if consumed 20 mL
	w/v	w/v	of syrup	of syrup	of syrup	of syrup	of syrup	of syrup
1	55	32	2.7	11	0.0350	0.0310	0.1420	0.1260
2	50.5	42	2.5	10.1	0.0310	0.0280	0.1260	0.1130
3	66.5	30	3.3	13.3	0.0370	0.0330	0.1740	0.1550
4	46	31	2.3	9.2	0.0250	0.0220	0.1010	0.0900
5	58	51	2.9	11.6	0.0380	0.0340	0.1540	0.1380
6	56.5	33	2.8	11.3	0.0330	0.0290	0.1330	0.1180
7	59.9	34	2.9	11.8	0.0310	0.0270	0.1240	0.1110
8	52	29	2.6	10.4	0.0330	0.0300	0.1350	0.1200
9	53.5	25	2.6	10.7	0.0360	0.0320	0.1440	0.1280
10	24	53	1.2	4.8	0.0140	0.0120	0.0550	0.0490
11	54	42	2.7	10.8	0.0290	0.0250	0.1160	0.1030
12	38	43	1.7	7.6	0.0191	0.0171	0.0764	0.0682
13	39	32	1.9	7.8	0.0211	0.0188	0.0844	0.0754
14	28.5	42	1.4	5.7	0.0197	0.0176	0.0817	0.0729
15	29	32	1.4	5.8	0.0206	0.0184	0.0838	0.0747

Therefore, the lethal doses of DEG ranges between 0.014 and 0.174 g/Kg or 14 mg–174 mg/Kg of body weight for the Argentinian episode while for the Haitian episode [15] the estimated mean was the 1.63 g/Kg in the range of 0.35 to 5.4 g/Kg in 32 patients and doses lower than 1.12 for 12 children [15]. The minimum limit published for the episode in Haiti is several times higher than the range reported in our study.

In sulfanilamide Massengill poisoning episode [7], lethal doses were registered in the range 1–2 g/Kg. This amount is notably higher than our results. We believe that the discussion about the DEG quantities ingested is very important in order to establish the lethal dose of DEG that according to our opinion will depend on different outbreak as it can be observed in the different mass poisonings the concentration of DEG in the contaminated product is different. In addition the ingested dose of the preparation should be considered, which will depend on the type of drug and the concentration for which it was prepared; the number of intakes and the time during which the DEG was incorporated until the onset of severe symptoms. The time elapsed before the start of the applied therapy could also have an impact on the different published doses. It should be noted that in the Argentinian case, the manifestations of intoxication appeared in a relatively short period of time from 24 to 48 hours after the syrup was ingested. The 5 mL and 20 mL of syrup were taken as lower and upper limits of real consumption in addition to the data on weight of the victims, while in other episodes they were calculated from Tables indirectly [3–5,14–15].

According to victim's relatives the symptoms begun after one to four portion intakes (the minimum of 5 mL arises from the recommended dose of propolis syrup). Once the symptoms started, the victims stopped ingesting the propolis syrup. This is the reason why we took two certain possibilities of ingestion (5 mL and 20 mL), which we further corroborated by the missing syrup in the original bottle.

Conclusion:

Up till now, about 20 cases of mass poisoning have been reported, with very similar symptoms and histopathological changes. Biotransformation follows a pathway in which the ether linkage is not cleaved, following a metabolic pathway leading to the formation of a highly nephrotoxic metabolite (HEAA and DGA) without production of oxalate crystals. Changes in internal environment parameters lead to serious or fatal consequences. Lethal doses seem to depend on each episode: circumstances of consumption, therapy applied and concentration of DEG in the drug vehicle. In our Argentinian episode the doses found were the lowest in line with the high percentages of DEG in the syrup.

Acronymns:

Diethylene glycol (DEG), HEAA (Ethoxy Hydroxy Acetic acid), Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT), Alcohol Dehydrogenase (ADH), Diglycolic Acid (DGA), Gamma Glutamyl Transpeptidase (GGT) and Lactate DeHydrogenase (LDH)

References

[1] Ferrari LA, Giannuzzi L. Clinical parameters, postmortem analysis, and estimation of lethal dose in victims of massive intoxication with diethylene glycol. Forensic Sci. Int 2005;153:45-51
[2] Existing Chemical Hazard Assessment Report. Diethylene Glycol. Australian Government. Commonwealth, Department of Health and Ageing, NICNAS, 2009 ISBN 978-0-9803124-6-1
[3] Wiener HL, Richardson KE. Metabolism of diethylene glycol in male rats. Biochem. Pharmacol. 1989;38: 539-541.
[4] Bastani P, Jammeh A, Lamar F, et al. Acute kidney injury among children likely associated with diethylene glycol–contaminated medications — The Gambia, June–September 2022. MMWR Morb Mortal Wkly Rep; 2023;72: 217–22.
[5] Schier J, Chang A, Kapil V. Medication-Associated Diethylene Glycol Mass Poisoning — A Preventable Cause of Illness and Death. N Engl J Med. March 30;2023; 388 (13): 1156–1157.
[6] Brent J. Lessons learned from yet another episode of diethylene glycol poisoning: it happened before and it happened again. JAMA Intern Med 2024; 174: 918–9.
[7] Leech PM. Elixir of sulfanilamide- Massengill. Chemical, pharmacological, pathologic and necropsy reports: preliminary toxicity reports on diethylene glycol and sulfanilamide. J. Am. Med. Assoc. 1937;109:1531-1539.
[8] Rentz ED, Lewis L, Mujica OJ, et al. Outbreak of acute renal failure in Panama in 2006: a case control study. Bull World Health Organ. 2008;86(10):749-756.
[9] O'Brien KL, Selanikio JD, Hecdivert C, Placide MF, Louis M, Barr DB, Barr JR, Hospedales J, Lewis MJ, Schwartz B, Philen RM, Victor SSt, Espindola J, Needhan LL, Denerville K. Epidemic of Paediatric Deaths from Acute Renal Failure Caused by Diethylene Glycol Poisoning. JAMA. 1998;279 1175-1180.
[10] Winek CL, Shingleton DP, Shanor SP. Ethylene and diethylene glycol toxicity. Clin Toxicol 1978;13: 297– 324.
[11] Snellings W, Snellings K, McMartin K, McMartin E, Banton M. Human health assessment for long□term oral ingestion of diethylene glycol. Regulatory Toxicology and Pharmacology 2017;87:Supplement 2:S1-S2.
[12] de Almeida Araújo, S, Dias Faria BC, Cunha Vasconcelos, et al. Renal toxicity caused by diethylene glycol: an overview. International Urology and Nephrology 2023; 55:2867–2875.
[13] Schep LJ, Slaughter RJ, Temple WA, Beasley DM. "Diethylene glycol poisoning". Clin Toxicol. 2009;47 (6): 525–35.
[14] Pandya SK. Letter from Bombay. An unmitigated tragedy". British Medical Journal. 1998;297 (6641): 117–9.
[15] Woolf A. The Haitian DEG tragedy. JAMA 1998;15;279 (15):1215-6.

Career Spotlight: Professor Hans Maurer

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Current Practice Areas/Industries:

Further development of new editions of three MS libraries:
Maurer HH, Meyer MR, Pfleger K, Weber AA. GC-MS library of drugs, poisons, and their metabolites. 6th ed. Weinheim: Wiley-VCH; 2023.
Maurer HH, Meyer MR, Helfer AG, Weber AA. Maurer/Meyer/Helfer/Weber MMHW LC-HR-MS/MS library of drugs, poisons, and their metabolites. 2nd ed. Weinheim, Germany: Wiley-VCH; 2023, in preparation.
Maurer HH, Meyer MR, Wissenbach DK, Weber AA. Maurer/Wissenbach/Weber MWW LC-MSn library of drugs, poisons, and their metabolites. 3rd ed. Weinheim, Germany: Wiley-VCH, 2024, in preparation.
Coauthor of other databases (e.g. Clarkes) and handbooks (e.g. Elsevier, NPS).
Teaching in national and international continuing and postgraduate education programs.
Editorial board member and reviewer of various scientific journals.

Education:
Study of Pharmacy at Saarland University in Saarbrücken (license to practice as pharmacist 1977), followed by study of Preclinical Medicine at Saarland University in Homburg.
After military service as medical officer at the German Navy, 1983 PhD in Pharmacology and Toxicology at Saarland University in Homburg resulting in the first edition of the GC-MS library in 1985.
Habilitation for Pharmacology and Toxicology in 1988.
1992 appointment as full professor and head of the Department of Experimental and Clinical Toxicology of Saarland University in Homburg (Saar).

Did you always hope to work in toxicology? What did you do in school to prepare yourself for those opportunities:

I did not think of toxicology at school because of missing information. My major focus was on chemistry but also on human biology and mathematics. As a consequence, I decided to study pharmacy combining at least chemistry and human biology. During my studies, I realized that pharmacology and toxicology would be ideal to specialize in during my PhD. First, I thought of working in a pharmaceutical company after my PhD, but my supervisor Prof. Karl Pfleger convinced me to continue in the academic field.

What work did you do following your initial undergraduate and postgraduate training and if applicable what trainings undertaken:

During my PhD and further studies, I completed the following postgraduate education:
Pharmacy Expert, Board-certified for Toxicology,
Pharmacy Expert, Board-certified for Pharmaceutical Analytics,
Clinical Toxicologist, certified by GTFCh,
Forensic Toxicologist, certified by GTFCh.

What do you most enjoy about your work:

The independency of a full professor, particularly concerning research projects. The wonderful collaboration with excellent PhD students. I'm proud that more than half of them stayed in our field, mostly in leading positions. Scientific discussion and development of friendships during national and international meetings.
The possibility to bring our field forward also by working for years in executive boards of scientific associations such as TIAFT, IATDMCT and GTFCh.

What piece of advice would you give to a current science/toxicology student/ early stage practitioner:

If you are looking for a PhD position check first the scientific outcome of the group e.g. in Pubmed and whether the PhD candidate has a chance to publish her/his work. This is important for a successful career in the field. Do a postdoc in another leading group to strengthen your expertise.

What might you do differently now that you have had all the experiences you have had if you had the chance to do it again:

Nothing ;-)

What do you like to do in your free time:

I'm a music and particularly opera lover. When travelling to meetings I also look for concert and opera performances so I could visit most famous opera houses around the word. Many TIAFT and IATDMCT friends joined me over the years.

After my retirement, I took over the presidency of the sponsor club of the Homburg Symphony Orchestra and I'm happy to organize for our members concerts with already successful young musicians and to award them with our Young Musician Award.

New synthetic opioids: the first three cases of etonitazepipne-related deaths ever reported in the literature, analysis of the National Early Warning System (SNAP)

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Keywords: New Psychoactive Substances, New Synthetic Opioids. Benzimidazole Opioids, Etonitazepipne

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The identification and circulation of New Psychoactive Substances (NPS) are closely monitored by international institutions, yet the illicit drug market continuously supplies new molecules and the total number of NPS is increasing. In 2022, 1165 NPS were totally identified at a global level of which about 20 new molecules were detected for the first time in the same year. In fact, this phenomenon is characterized by the continuous variation in the type of substances [1].
The pharmaco-toxicological properties of NPS are generally not scientifically evaluated when a new molecule appears on the illegal market and user experience is often the only source of information. As a result, unexpected NPS-related side effects causing intoxication and death are reported with a wide range of nonspecific signs [2,3].

In response to this global public health issue, national governments, research and medical institutes as well as international agencies have joined efforts and combined local surveillance networks into a comprehensive system to monitor the development of the NPS market.

Since 2016, the Italian National Institute of Health (Istituto Superiore di Sanità, ISS) was entrusted by the Anti-drug Policies Department of the Presidency of the Council of Ministers (Dipartimento delle Politiche Antidroga, DPA) as the coordinator of the National Early Warning System (Sistema Nazionale di Allerta Precoce, SNAP). Therefore, SNAP was initially set up with biotoxicological, clinico-toxicological and law enforcement expertise. Within this network, the Central Directorate for Antidrug Services (DCSA) is responsible for coordinating police forces regarding drug seizures. The forensic toxicology unit of the Sapienza University of Rome, was responsible for: processing information from suspected NPS exposed cases, developing analytical methods to detect the latest molecules and conducting metabolic studies to identify new markers of exposure. The Poison Center in Pavia was responsible for the clinical-toxicological aspects, handling the toxicological interpretation of samples from NPS-related cases in Italy.

Then, in 2020, SNAP was further developed with two more operative units with bio-toxicological expertise: the Department of Biomedical Science and Public Health of University “Politecnica delle Marche” and the “Centro Regionale Qualità Laboratori” of Health Department of Sicily Region. Furthermore, the network counts more than 200 collaborative centres that have joined SNAP, such as law enforcement agencies, analytical toxicology laboratories, forensic toxicology laboratories, poison control centres, health systems, research institutes and universities and drug regulatory authorities.

Following the European guidelines on Early Warning Systems, the SNAP system is structured as an input-output information flow. As the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) issues a new communication on NPS in Europe, the ISS transmits the information through an online platform (snap.iss.it) as a new document that is shared to facilitate communication and information exchange. Afterwards, the information produced and managed is evaluated from a public health and social risk perspective to perform the linked risk assessment. Depending on the severity of the event and the expected public health consequences, grade I, II or III "Alerts" are issued to provide timely sensitive information on NPS that may pose a threat to public health on the territory. To improve monitoring, analysis and response to such events, the SNAP annually distributes to the network the pure chemical standards of the most recent NPS monitored by the EMCDDA.
Furthermore, it contributes to the implementation of analytical techniques for the identification of assigned compounds in biological matrices [4].

Over the past five years, the collaborative centres have reported about 365 events related to 169 NPS detected in Italy, representing 62% of all entry reports reviewed by the ISS. Synthetic cathinones were the most reported substances (28%), followed by cannabinoids (14%), opioids (12%) and arylcyclohexylamines (12%). Substances belonging to psychoactive tryptamines, benzodiazepines, piperazines and others were identified less frequently. Among these substances, there were 49 new emerging NPS, mainly synthetic cathinones (35%), synthetic cannabinoids (18%) and synthetic opioids (10%).

The first New Benzimidazole opioid related death:

The current illicit opioid market is characterized by a supply of new, extremely potent synthetic compounds used alone or in combination with other drugs of abuse (DoA) [4]. Benzimidazole opioids (BO) represent the newest DoA, being up to 100 times more potent than morphine [5]. The newest BO, etonitazepipne, has been closely monitored by the EMCDDA since 2022 due to its severe toxicity and rapid spread in the European illicit market.

Following the first identification in the United States in patients with suspected nonfatal opioid overdose in 2021 [5], etonitazepipne was detected in plasma (1.21 ng/mL) and urine (0.51 ng/mL) samples of nonfatal opioid-related intoxication cases in Belgium in 2022 [6]. Until then, no toxicological analytical data on fatal intoxication cases were reported. This could be due to the unavailability of reference standards in pharmaco-toxicological laboratories, or to the lack of an effective analytical method allowing the detection of the negligible amount of benzimidazole opioids in biological matrices able to generate psychoactive effects. Indeed, last generation hyphenated techniques (such as gas chromatography or liquid chromatography coupled with tandem mass spectrometry or high-resolution mass spectrometry) may be necessary for the detection and determination of such substances [7].

We describe here the first three cases of etonitazepipne-related deaths ever reported, that were identified thanks to the SNAP network and promptly reported to the EMCDDA in 2022. In July 2022, the first case of fatal intoxication involved a 42-year-old female, found dead in her apartment in advanced decomposition status. Since a bag labelled “etonitazepipne” was found next to the corpse, the ISS was alerted through the SNAP network to provide analytical confirmation of the substance. The available post-mortem matrices were screened and then confirmed by liquid chromatography coupled to high-resolution mass spectrometry, substantiating etonitazepipne consumption. Hence, a grade III alert was rapidly issued and communicated across Italian territory to warn the authorities and the health personnel of this new harmful NPS arrival on the illegal market market. As a consequence, the University Politecnica delle Marche attentively monitored the regional situation, advising the ISS of a suspicious overdose series that occurred in the same geographical area in the same period. Afterwards, the attention was raised on two other fatalities involving two males aged 40 and 43 years.

The analytical protocol:

Biological matrices were collected during autopsies and processed as follows: A volume of urine (500 µL), gastric contents (500 µL) and vitreous humor (200 µL) were liquid/liquid extracted under basic (pH=10) and acidic (pH=3) conditions with 1.5 mL of chloroform: isopropanol (9:1, v/v). Then, the extraction solvent was collected from both aliquots of the same sample and dried under a gentle flow of nitrogen at room temperature. Also, a volume of 100 µL of whole blood was deproteinated with 200 µL of methanol: acetonitrile (50:50 v/v) and ultracentrifuged at 14,000 rpm at 4°C for 10 min. The supernatant was filtered through the Phree™ cartridge (Phenomenex, Italy) and dried under a gentle nitrogen flow. Before injection into the liquid chromatograph, the samples were reconstituted in 100 µL of mixed mobile phase A (ammonium formate 2mM, 0.1% formic acid) and mobile phase B (ammonium formate 2mM in methanol:acetonitrile 50:50, 0.1% formic acid) (80:20 v/v) and 10 µL were injected. The samples were derivatized with a silylation reagent at 70°C for 30 minutes for the gas chromatographic separation for the quantification of drug of abuse detected in the screening analysis.

The LC-MS/HRMS analyses were performed on an Ultimate 3000 liquid chromatography coupled to a high-resolution QExactive Focus orbitrap tandem mass spectrometer (Thermo Fisher Scientific, MA, USA) equipped with a HESI ionization source (Thermo Fisher Scientific, MA, USA). The analytes were chromatographically separated through an Accucore™ phenyl Hexyl column (100 x 2.1 mm, 2.6 µm, Thermo Fisher Scientific, USA) maintained at 40 °C for the entire run. Gas chromatographic instrumentation was an Intuvo (Agilent Technologies, CA, USA) coupled to a single quadrupole mass spectrometer (Agilent Technologies, CA, USA).

The screening analyses were performed according to previously published methods [7] and a dedicated method was developed and validated in LC-MS/HRMS to detect and quantify etonitazepipne in all matrices.

The GC-MS quantitative analyses were performed on an Intuvo™ (Agilent, CA, USA) gas chromatograph coupled to a single quadrupole mass spectrometer 5977B (Agilent, CA, USA) interfaced by an electronic impact source operating at 70eV. The chromatographic separation was carried out through a DB-5MS ultra inert column (30m, 0.25 mm x µm) using helium as the gas carrier. Previously validated in-house methods were applied for illicit opioids, cocaine and related metabolites quantification in all the available matrices.

Toxicological analysis revealed the presence of only etonitazepipne in the vitreous humor (1.07 ng/mL), peripheral blood (traces) and gastric contents (14.7 ng/mL) of the 42-year-old woman, a former heroin addict, found dead in her apartment in a state of advanced decomposition. In the case of the 40-year-old man, who was addicted to heroin and cocaine, etonitazepipne was quantified only in urine (1.62 ng/mL) along with 6-monoacetylmorphine, morphine, cocaine and benzoylecgonine while the peripheral blood showed only traces of morphine. In the last case, involving a 43-year-old cocaine addict, etonitazepipne was found in peripheral blood (traces) and urine (0.48 ng/ml) along with cocaine and benzoylecgonine.

The toxicological results:

In the case of the 42-year-old female, etonitazepipne was considered as the main cause of death, as there was no opioid tolerance and there were no autopsy findings to support a different cause of death. In the other two cases, the unique role of etonitazepipne could not be determined but it may have synergistically acted with heroin and cocaine. Moreover, it cannot be established whether heroin and cocaine were consumed in combination with etonitazepipne or it was added as an adulterant during the manufacturing process. However, for the first time, etonitazepipne was detected in postmortem biological matrices, presumably playing an important role in determining death albeit through different mechanisms.

Notably, the three deaths share minimal benzimidazole opioid concentrations in blood and/or urine, highlighting the high toxicity of etonitazepipne and consequently the high risk of related overdose [8].

Through these reports, the Ministry of Health was alerted and the substance was scheduled as "illegal", according to national anti-drug law. This demonstrates the value and efficiency of SNAP.

Acronymns:

Istituto Superiore di Sanità (ISS), New Psychoactive Substances (NPS)
European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Sistema Nazionale di Allerta Precoce (SNAP), Central Directorate for Antidrug Services (DCSA)

References:

[1] UNODC, "World Drug Report 2023," 2023. Accessed: Oct. 09, 2023. [Online]. Available: https://www.unodc.org/unodc/en/data-and-analysis/wdr-2023_booklet-2.html. Accessed 22/06/24

[2] Emcdda European Drug Report 2020: Trends and Developments. 10.2810/123451. www.emcdda.europa.eu (24 February 2021). Accessed 22/06/24

[3] UNODC (United Nation Office on Drugs and Crime) (2020) Word Drug Report- Executive summary, impact of COVID-19, policy implications. Accessed 22/06/24

[4] Di Trana A, Pichini S, Pacifici R, Giorgetti R, Busardò FP. Synthetic Benzimidazole Opioids: The Emerging Health Challenge for European Drug Users. Front Psychiatry. 2022 Mar;13:858234

[5] Vandeputte MM, Krotulski AJ, Walther D, Glatfelter GC, Papsun D, Walton SE, Logan BK, Baumann MH, Stove CP. Pharmacological evaluation and forensic case series of N-pyrrolidino etonitazene (etonitazepyne), a newly emerging 2-benzylbenzimidazole 'nitazene' synthetic opioid. Arch Toxicol. 2022 Jun 1 ;96(6):1845–63.

[6] Marchei E, Ferri MA, Torrens M, Farré M, Pacifici R, Pichini S, Pellegrini M. Ultra-High Performance Liquid Chromatography-High Resolution Mass Spectrometry and High-Sensitivity Gas Chromatography-Mass Spectrometry Screening of Classic Drugs and New Psychoactive Substances and Metabolites in Urine of Consumers. Int J Mol Sci. 2021 Apr 2;4000-4021.

[7] EMCDDA. New psychoactive substances: 25 years of early warning and response in Europe. 2022. Accessed 22/06/24

[8] Di Trana A, La Maida N, Froldi R, Scendoni R, Busardò FP, Pichini S. The new synthetic benzimidazole opioid etonitazepipne: an emerging fatal harm and a challenge for laboratory medicine. Clin Chem Lab Med. 2023; 61(10): e200–e202

Snake Venom as a Recreational Substance of Abuse in India

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Keywords: Snake, Snake Venom, India, Recreational Use, Psychoactive Effects, Neurotoxin, Envenoming, Abuse, Toxicology

India is a tropical country, home to diverse flora and fauna. Traditionally, there has been a diverse range of psychoactive substances used in the country. Some of the well-known and studied psychoactive substances include psilocybin mushrooms, marijuana, cocaine, 3,4-methylenedioxymethamphetamine (MDMA) and lysergic acid diethylamide (LSD). There is a substantial body of literature on these substances including their use and abuse, psychotropic effects and potential to cause death [1]. People who actively seek out such experiences with psychoactive substances are known as "psychonauts". One unique and unusual psychoactive substance used extensively in India is snake venom. The use of snake venom is nothing new in India, but there is little to no literature that describes its use or effects [2]. The toxicology of snake venom and its recreational consumption will be described below as well as the native snake species used for this purpose, the typical modes of administration, patterns of use and misuse with the aim to attempt to gain an insight into the motivations behind such experimentations.

Snakes have been of great importance in different cultures around the world. In ancient Greek culture, snakes were seen as a symbol of renewal and rebirth due to their ability to shed their skin [3]. Greeks also believed that snake venom had healing properties; a snake always accompanied Asclepius, the Greek god of medicine. In many ancient folklores, snake oil was used to treat rheumatism, help with insect bites, and even increase virility [4]. In some Indian tribes, consuming small doses of snake venom during childhood is thought to provide immunity against snake bites [5].

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Figure 1: Russell's Viper, Daboia russelii [6]

Figure 2: Common Krait, Bungarus caerules [7]

Figure 3: Indian Saw-Scaled viper, Echis carinatus [8]

Figure 4: Indian Cobra, Naja naja [9]

There are thousands of species of snakes found in India due to the biodiversity of the country. The big four, the most known venomous snakes in India are named above.

Table 1: Various types of toxins in four venomous snakes in India. [12]

Venom properties \ Common Name, Species	Russell's Viper, *Daboia russelii*	Common Krait, *Bungarus caeruleus*	Indian Spectacled Cobra, *Naja naja*	Indian Saw-scaled Viper, *Echis carinatus*
Average Venom Quantity	130 to 250 mg (dry weight)	8 to 20 mg (dry weight)	169 to 250mg (dry weight)	20 to 35 mg (dry weight)
Venom Neurotoxin	Presynaptic neurotoxins	Pre- & post-synaptic neurotoxins	Postsynaptic neurotoxins	Not present
Venom Myotoxin	Systemic myotoxins	Not present	Probably not present	Not present
Venom Procoagulants	Mixture of procoagulants	Not present	Probably not present	Mixture of procoagulants
Venom Anticoagulants	Possibly present	Not present	Probably not present	Possibly present
Venom Haemorrhagins	Zinc metalloproteinase	Not present	Probably not present	Zinc metalloproteinase
Venom Nephrotoxins	Possibly present	Not present	Probably not present	Possibly present
Venom Cardiotoxins	Probably not present	Not present	Probably present	Probably not present
Venom Necrotoxins	Possibly present	Not present	Possibly present	Possibly present
Venom Other	Unknown	Not present or not significant	Not present or not significant	Unknown

The big four venomous snakes of India account for most of the envenoming cases reported. A 2008 study on envenoming and deaths reported that India had the highest number of snake-related fatalities worldwide with 81,000. Envenoming cases and nearly 11,000 snake-related deaths annually [11]. Unfortunately, the authors of that study mentioned the overall number of deaths due to snake bites without specific details about any deaths associated with recreational use.

A literature survey which spanned three decades on the recreational use of snake venom in India demonstrated that most snake venom users were male. The lack of information about women users of recreational snake venom in India is due to the rarity of the practice, societal stigma, underreporting and gender biases in research. The mean average value of men's age is 29 and the median value is 25. They come from different socio-economic backgrounds. Their educational qualifications ranged from having no formal education to being graduates.

When questioned about their motives for using snake venom, most participants cited reasons such as seeking a heightened sense of euphoria, stress relief, curiosity and tolerance to other drugs. The species of snake reported to be most often used were the cobra, followed by the rat snake and an unspecified green snake. The reported symptoms experienced by users included grandiosity, a sense of well-being and increased arousal [13-19].

Snake venom is typically used via a rather unique method of intake compared to other common psychedelic drugs. Introducing the venom into the body involves getting bitten by a venomous snake resulting in the delivery, of the snake's neurotoxin. In India, snake charmers, individuals skilled in handling venomous snakes, often allow themselves to be bitten in exchange for monetary compensation [20]. The preferred bite locations are typically the tongue, lips, fingers, toes or soles. In places known as 'Snake Dens', the snake charmer taps over a snake's head and in response, the snake bites. Another way to intake snake venom is by injecting the extracted snake venom directly into the body but this is a costly way of getting high for Indians [21].

Figure 5: Snake venom extracted from a live snake. [22]

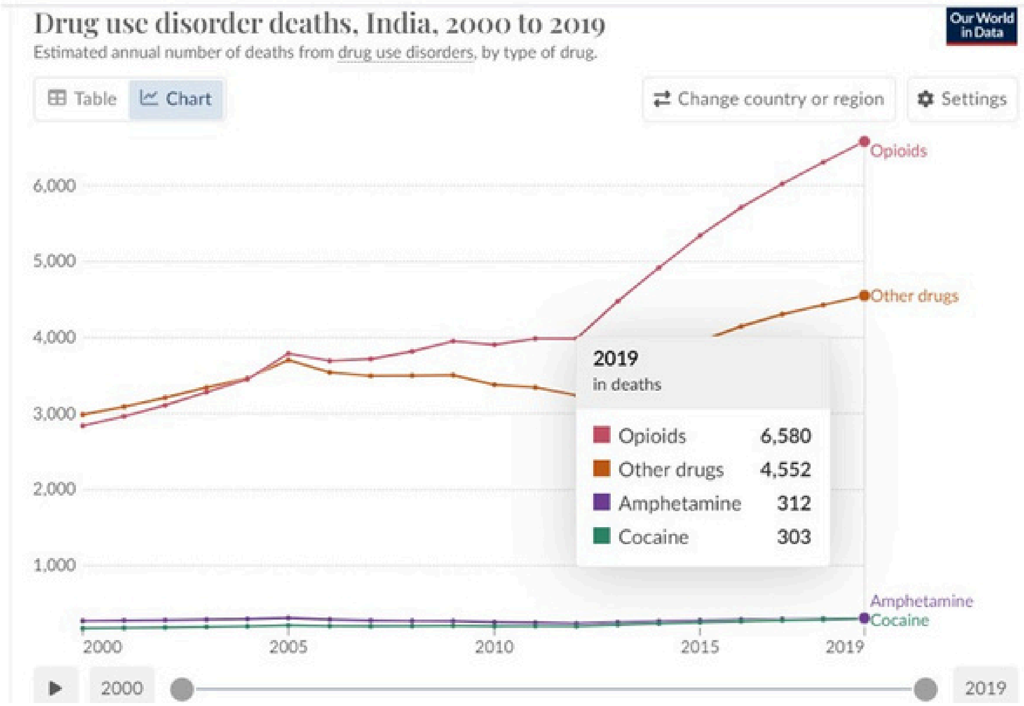
To prepare snake venom for oral consumption, the venom is prepared by converting it into a dry powder or tablet. The venom is lyophilised (freeze-dried) by mixing it with cryoprotectants like sucrose or mannitol followed by grinding [23]. This powder or tablet can be mixed with other intoxicating substances such as ethanol to add an extra 'kick' for the user. Often, cobra venom powder is sold at raves in India under the names 'K-72' and 'K-76' [24].

Snake venoms are complex mixtures of bioactive compounds, primarily proteins and peptides. Some components of snake venom affect the nervous system and alter mood, perception or consciousness. Neurotoxins like α-Bungarotoxin and dendrotoxin interfere with nerve signalling by blocking acetylcholine receptors at neuromuscular junctions and blocking potassium channels affecting nerve transmission. Slow-reacting substances including prostaglandins and bradykinin-potentiating peptides increase vascular permeability and enhance blood flow throughout the body inducing hypotension [25]. Upon entering the body, several of the chemicals found in snake venom aid in the release of serotonin and induce euphoria.

It is well understood that the venom's absorption, distribution, metabolism and elimination are species-specific. A recent study showed that snake venom's average half-life is approximately 10 hours. However, each user experiences the venom's half-life and pharmacokinetics differently while pretty much everyone experiences its psychedelic effects in a similar time frame [26]. This phenomenon makes users take multiple doses, which often results in death.

The desire to recreationally use snake venom as a psychotropic substance can be explained by understanding the chemical reactions happening in the brain once the venom is consumed. The venom's neurotoxin reaches the brain and competes with acetylcholine to bind to nicotinic acetylcholine receptors. This bond causes muscular paralysis and centrally mediated opiate-independent analgesia [27]. The snake venom, once ingested, is quickly distributed throughout the body but its metabolism is, significantly slower. The extent and duration of the effects of snake venom are dependent on the mode of its administration. If injected directly into the vein, the venom spreads rather quickly but remains in the body for a shorter period. In contrast, the venom takes longer to distribute when injected under the skin or into the muscle and it is retained by the body for an extended period [26].

Figure 6: Drug us disorder deaths, India 2000 to 2019. [31]



Currently, there are two available explanations for the psychoactive effects experienced by users. The first is based on the venom's neurotoxic properties. Some low molecular peptides can and do cross the blood-brain barrier and are responsible for the psychoactive effects. Studies have shown the use of various components of snake venom are used as opioid-independent analgesia [28]. The second focuses on the induced altered physical states. These findings cannot be applied to explain the psychoactive effects of snake venom. The psychoactive effect may be due to the influence of multiple substances of abuse at the time of the bite, the intimation by peers or snake charmer or feeling rewarded when seeking adrenaline-releasing measures. This is true when the specific active compound responsible for the psychoactive effects in certain fauna has not been identified [29].

The rising trend of snake venom abuse, especially from the cobra species, is a concerning phenomenon in the pursuit of psychoactive effects via perilous means. The familiarity of handling cobras in India dates to British rule, known as the 'cobra effect' [30]. This makes cobras the most popular species to be abused for recreational use.

Despite reports highlighting feelings of well-being and sedation, the risks associated with snake venom intoxication far outweigh any perceived benefits. According to the World Health Organization's Global Health Estimates, deaths related to the use of illicit drugs such as opioids, amphetamines, cocaine and other substances have been thoroughly documented in India from 2000 to 2019 [31]. The lack of substantial medical literature on the subject, particularly in India, highlights the need for increased awareness and prevention efforts. The public must understand the grave risks associated with using snake venom as a means of achieving a high. Not only is there no scientific evidence to support the notion that it produces the desired effects. Its excess ingestion causes peripheral neuromuscular blockade leading to respiratory paralysis and even death [32]

The limited information on the psychedelic effects of snake bites due to cultural and traditional limitations is a major hindrance to understanding this issue. Medical literature on snake venom in India primarily focuses on creating anti-venom to prevent mortality caused by snake bites, which overshadows the recreational use of these substances in public.

Fortunately, the increase in discussions on novel psychoactive substances and media coverage is shedding light on these hidden substances with abuse potential. In May 2024, a recent case of snake venom abuse came to light thus making headlines [33]. Greater awareness will hopefully result in more research and can help create a greater understanding of snake venoms as a matter of public safety in India and beyond.

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Acronmyns:

3,4-methylenedioxymethamphetamine (MDMA)
Lysergic Acid Diethylamide (LSD)

References:

[1] Passie T, Brandt S. Self-Experiments with Psychoactive Substances: A Historical Perspective. Handbook of Experimental Pharmacology. 2018; Vol (252). doi: 10.1007/164_2018_177
[2] Jadav D, Shedge R, Meshram V, et al. Snake Venom – An unconventional recreational substance for psychonauts in India. Journal of Forensic and Legal Medicine. 2022, Volume (91).
[3] Ramoutsaki I, Haniotakis S, Tsatsakis A. 'The snake as the symbol of medicine, toxicology and Toxinology'. Veterinary and Human Toxicology. 2000, Volume 42(5):306 - 308.
[4] Starin D. The false promise of Snake wine in Southeast Asia. Geographical.co.uk [Internet]. 2021. Available at: https://geographical.co.uk/culture/the-false-promise-of-snake-wine-in-southeast-asia.
5] Senthilkumaran S, Balamurugan N, Menezes R, et al. 'Repeated snake bite for recreation: mechanisms and implications'. International Journal of Critical Illness and Injury Science. 2013, doi: 10.4103/2229-5151.119202
[6] Chawla A. Daboia russelii Russel's Viperin CME Dapodi campus[Photograph] 2010. print: colour.
[7] Chiplunkar J. Bungarus caeruleus, the common krait. [Photograph] 2011. print: colour.
[8] Kuveskar S. Saw-scaled Viper (Echis carinatus) in Mangaon, Raigad, Maharashtra, India [Photograph] 2014. print: colour.
[9] Jolle~commonswiki Assumed (No machine-Readable Source Provided, Based on Copyright Claims). Naja naja, the Indian cobra [Photograph] 2009. print: colour.
[10] Mukherjee A. 'Green Medicine as a Harmonizing Tool to Antivenom Therapy for the Clinical Management of Snakebite: The Road Ahead'. Indian Journal of Medical Research. 2012, 136(1): 10-12, PMCID: PMC3461710.
[11] Kasturiratne A, Wickremasinghe R, De Silva N. The Global Burden of Snakebite: A Literature Analysis and Modelling Based on Regional Estimates of Envenoming and Deaths'. PLoS Medicine. 2008, doi: 10.1371/journal.pmed.0050218
[12] Toxinology Department. Clinical Toxinology Resources. The University of Adelaide, Australia. 2016. Available at: http://toxinology.com/fusebox.cfm?fuseaction=main.snakes.search (Accessed: 21/03/2024).
[13] Katshu M.Z.U.H, Dubey I, Khess C.R.J, et al. 'Snake Bite as a Novel Form of Substance Abuse:Personality Profiles and Cultural Perspectives'. Substance Abuse. 2011; 32:43–46, doi: 10.1080/08897077.2011.540482
[14] Pradhan P.V, Shah L.P, Ghodke P.R. 'Snake Venom Habituation in Heroin (brown sugar) Addiction: (report of two cases)'. Journal of Postgraduate Medicine. 1990; Vol 36(4):233 - 234, PubMed ID: 2132253.
15] Senthilkumaran S, Shah S, Balamurugan N, et al. 'Repeated Snake Bite for Recreation: Mechanisms and implications'. Int J Crit Illn Inj Sci. 2013; 3(3):214 - 216.
[16] Krishnamurthy K, Braganza W. 'A Unique Case of Substance Abuse'. Muller Journal of Medical Sciences and Research. 2013; 4(1):23-25, doi: 10.4103/0975-9727.112269
[17] Umate M.K, Khot P, Salukhe R, et al. 'Snake venom Abuse in Rave Parties: A Case Report'. Indian Journal of Mental Health. 2015; 2:227-9.
[18] Talwar D, Kumar S, Acharya S. 'A Curious Case of Snake Venom Addiction as an Alcohol De-Addiction Tool: Pain for Gain?'. Cureus. 2021; 13(11), doi: 10.7759/cureus.19996
[19] Ram D. 'Recreational Use of Snake Venom, an Emerging Trend in India – A Case Report and Review of Reports'. Scientific Scholar. 2022; 65(4):242-244, doi: 10.25259/IJPP_303_2021

[20] Das S, Barnwal P, Maiti Y, et al. 'Addiction to Snake Venom'. Substance Use and Misuse. 2017, Vol 52(8):1104-1109, doi: 10.1080/10826084.2016.1272614
[20] Das S, Barnwal P, Maiti Y, et al. 'Addiction to Snake Venom'. Substance Use and Misuse. 2017, Vol 52(8):1104-1109, doi: 10.1080/10826084.2016.1272614
[21] Vijay K, Pravir B. 'Snake Venom -The New Range to Get High!'. Journal of the Indian Society of Toxicology. 2012, Volume 8(1):46-48, Online ISSN: 0973-3566

[22] Swart J, Jimenez P, Singh A, et al. A Snakebites into Cellophane Covering the Top of a Shot Glass and Releases its Venom into the Glass [Photpgraph]. The Process of Collecting Snake Venom to Produce Life-saving Anti-venom. 2023. Available at: https://www.businessinsider.com/photos-process-of-developing-anti-venom-snakebites-2023-11. print: colour.

[23] Chakma J.K, Menon J.C, Dhaliwal R.S. 'White Paper on Venomous Snakebite in India'. Indian Journal of Medical Research. 2020, 152(6): 568-574, doi: 10.4103/ijmr.IJMR_3377_20. (Accessed 21/03/2024).

[24] Singh S. New Party Drug ‘Snakebite’ Worries Officials. Sundayguardianlive.com [Internet]. 2016. Available at: https://sundayguardianlive.com/investigation/4180-new-party-drug-snakebite-worries-officials (Accessed: 21/03/2024).

[25] Messadi E. 'Snake Venom Components as Therapeutic Drugs in Ischemic Heart Disease'. Biomolecules. 2023. 13(10). doi: 10.3390/biom13101539

[26] Sanhajariya S, Duffull S, Isbister G. 'Pharmacokinetics of Snake Venom'. Toxins (Basel). 2018. 10(2):73. doi: 10.3390/toxins10020073

[27] Koh D.C.I, Armugam A, Jeyaseelan K. 'Snake Venom Components and Their Applications in Biomedicine’. Cellular and Molecular Life Sciences CMLS. 2006, 63:3030–3041. doi:10.1007/s00018-006-6315-0

[28] Xiong Y, Wang W, Pu X. ' Preliminary Study on the Mechanism of Using Snake Venoms to Substitute for Morphine'. Toxicon. 1992. 30:67-568.

[29] Orsolini L, Michela C, Duccio P, et al. 'Psychedelic Fauna for Psychonaut Hunters: A Mini Review'. Frontiers in Psychiatry. 2018. Volume 9, Article number 153, doi: 10.3389/fpsyt.2018.00153

[30] Horst S, 'Der Kobra-Effekt. Wie Man Irrwege der Wirtschaftspolitik Vermeidet. ['The Cobra Effect. How to Avoid Wrong Paths in Economic Policy']. Munich: Deutsche Verlags-Anstalt, ISBN 3421055629; 2001. German.

[31] Statical Representation of Drug Use Disorder Deaths, India, 2000 to 2019, Estimated Annual Number of Deaths from Drug Use Disorders, by Type of Drug, WHO, Global Health Estimates, 2020, URL: https://ourworldindata.org/illicit-drug-use. (Accessed: 21/03/2024).

[32] Shukla L, Reddy S.S., Kandasamy A, et al. 'What Kills Everyone, gives a High for Some—Recreational Snake Envenomation'. Asian Journal of Psychiatry. 2017; Vol 25:106-108, doi: 10.1016/j.ajp.2016.10.003

[33] ETV Bharat English Team, 'Elvish Yadav Snake Venom Case: YouTuber Contacted Snake Charmers Through Virtual Number, Police Say'. Etvbharat.com [Internet]. 2024, URL: https://www.etvbharat.com/en/!state/elvish-yadav-snake-venom-case-youtuber-used-virtual-number-to-contact-snake-charmers-charge-sheet-enn24052301320.

Recent Evidence of the Presence of Xylazine in the North of Scotland

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Keywords: Xylazine, Designer Drugs, Scotland

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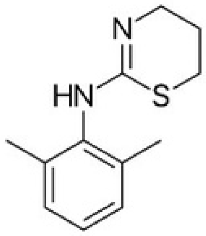
Scotland has the unenviable statistic of most drug fatalities per capita in Europe, with opiates, benzodiazepines, methadone and cocaine being the most frequently encountered drugs in these deaths [1,2]. It has also not escaped the emergence of novel psychoactive compounds over the last 10-15 years. These compounds, most notably designer benzodiazepines such as etizolam, flubromazepam, flualprazolam, flubromazolam and now currently bromazolam often feature in Scottish drug deaths. However, it is the opioid epidemic in the US that is ringing alarm bells in the North of Scotland. Since 2023, several 2-benzyl benzimidazole opioid ('nitazene') compounds have been detected in post-mortem casework alongside bromazolam and opiates [3,4] This is very similar to the pattern currently seen in the US, but now added to this mix of drugs is xylazine, an alpha 2-adrenoceptor agonist animal tranquiliser with sedative, analgesic and muscle relaxant effects [5,6]. It has emerged as an adulterating agent in a variety of recreational drug preparations, including cocaine, heroin, fentanyl and combinations of these substances, commonly referred to as 'Tranq dope' or just 'Dope', (Figure 1) and has been implicated as a contributing cause of death in several cases both alone and in combination with other drugs. It is now currently being increasingly observed alongside fentanyl and nitazene compounds in forensic casework, prompting Public Health Scotland to issue a recent Rapid Action Drugs Alert and Response (RADAR) alert [7,8]. In Grampian and Shetland, eight post-mortem cases from October 2023 to June 2024 have been found to be positive for xylazine, representing its presence in 7% of the drug-related fatalities during that period. Seven of the eight cases were also found to be positive for bromazolam, itself being found in 25% of our current drug-related deaths. Xylazine has not been found in isolation in any of these cases: the notable detected recreational drugs associated with these cases being cocaine (five cases), diazepam (7 cases), methadone (four cases), morphine (5 cases), gabapentinoids (two cases) and cannabinoids (four cases). Other drugs detected amongst the cases with xylazine included alprazolam, amitriptyline, buprenorphine, citalopram, etizolam, fentanyl (one case), ketamine, levetiracetam, olanzapine, paracetamol, phenacetin and tramadol. Intriguingly, only three of the eight cases were also found to contain nitazenes: one case with isotonitazene, 5-aminoisotonitazene and metonitazene present and two cases with both metonitazene and protonitazene present. Although the pattern of drugs in these cases with xylazine detected resembles findings in the US as they do not appear to directly mirror its co-detection alongside fentanyl. In only one case, fentanyl was also detected whereas in the US it appears fentanyl or a fentanyl derivative is associated in most cases of seized Tranq dope samples [9].

Figure 1: Xylazine (recreationally known as Tranq dope)



Source: Xylazine on PubChem, Public Domain, https://commons.wikimedia.org/w/index.php?curid=45601497 Accessed 020724

It is possible that other non-screened for 'designer fentanyl compounds' could be involved in these cases, but screening with high resolution accurate mass spectrometry following a solid phase extraction method on post-mortem blood for basic and neutral drug compounds did not indicate the presence of any further unconfirmed compounds that might have indicated their presence in the samples. Xylazine concentrations in the post-mortem blood of the eight cases range from 0.5 ng/mL - 216.8 ng/mL (average 42.3 ng/mL, median 3.5 ng/mL). The observed levels are similar to those reported in a case from 2023 in the UK with a post-mortem xylazine level of 38 ng/mL and levels of 7.4 and 8.3 ng/mL observed by Reyes et al. (2024) in the work-up of a high resolution mass spectrometric method [10,11]

Although the presence of xylazine in these cases was not taken in isolation regarding the toxicological element involved in the cause of death, it was implicated as a contributing cause in combination with other drugs. The importance of its presence in current post-mortem toxicology testing should not be understated. Up till now, detection of xylazine in post-mortem casework appeared to be confined to North America but its recent presence in fatal drug overdoses in the North of Scotland, in addition to a case report in 2023 from the UK, should serve as a warning of its emergence in drug related deaths involving illicit benzodiazepines, cocaine and opioids and its inclusion in illicit cocaine and opioid preparations being sold in the UK and Europe.

References:

[1] Christie B. Drug Deaths Fall in Scotland but Major Problems Persist. BMJ, 2023; 382; 1947. doi: https://doi.org/10.1136/bmj.p1947 (Published 23 August 2023).

[2] National Records of Scotland. Drug-related Deaths in Scotland in 2022. 22 Aug 2023. https://www.nrscotland.gov.uk/files//statistics/drug-related-deaths/22/drug-related-deaths-22-report.pdf (Accessed 05/07/24)

[3] Public Health Scotland Nitazenes Alert. Published 24 January 2023; updated 20 December 2023. https://publichealthscotland.scot/publications/rapid-action-drug-alerts-and-response-radar-alerts/radar-nitazenes-alert-2023/ (Accessed 01/07 24).

[4] Cowan D. Dangerous Nitazene Drugs linked to Rise in Deaths. BBC News article. https://www.bbc.co.uk/news/articles/c4nn25qzel30#:~:text=The%20National%20Crime%20Agency%20has,into%20street%20drugs%20like%20heroin (Accessed 01/07/ 24).

[5] Friedman J, Montero F, Bourgois P et al. Xylazine Spreads Across the US: a Growing Component of the Increasingly Synthetic and Polysubstance Overdose Crisis. Drug Alcohol Depend, 2022; 233: article 109380.

[6] Malaca S, Pesaresi M, Kapoor A et al. Pharmacology and Toxicology of Xylazine: Quid Novum? Eur Rev Med Pharmacol Sci, 2023; 27(15): 7337-7345. DOI: 10.26355/eurrev_202308_33305.

[7] Center for Forensic Science Research & Education (CFSRE) - Xylazine: A Toxic Adulterant Found in Illicit Street Drugs. https://www.cfsre.org/images/content/reports/public_alerts/2020.10.20.Public-Alert_Xylazine-Final.pdf (Accessed 26 June 2024).

[8] Public Health Scotland Xylazine Alert. Published 09 May 2024; updated 10 May 2024. https://publichealthscotland.scot/news/2024/may/radar-alert-issued-on-new-synthetic-drug-xylazine/ (Accessed 01/07/24).

[9] The Center for Forensic Science Research & Education (CFSRE) Drug Checking Quarterly Report (Q1-2 2023) (2023) Philadelphia, Pennsylvania, USA. https://www.cfsre.org/images/content/reports/drug_checking/2023_Q1_and_Q2_Drug_Checking_Quarterly_Report_CFSRE_NPS_Discovery.pdf (Accessed 07/07/ 24).

[10] Rock KL, Lawson AJ, Duffy J et al. The First Drug-related Death Associated with Xylazine Use in the UK and Europe. J Forensic Leg Med, 2023; 97: 102542. https://doi.org/10.1016/j.jflm.2023.102542

[11] Reyes AG, Krotulski A, Oetjen K and Chay L. Detection of Xylazine in Whole Blood Samples using High Resolution Mass Spectrometry. SCIEX Technical note MKT-29707-A (Accessed 01/07/24).

From Plant to Vapor and Jellies: Understanding Synthetic Cannabinoids, Delta-8-Tetrahydrocannabinol and Their Place in the Cannabis World of Vaping and Edibles

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Keywords: Cannabis, Synthetic Cannabinoids, Delta 8-THC, Vaping, Cannabis Edibles

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New Psychoactive substances (NPS) is a broad term used to describe various drugs initially designed to imitate or replace traditional drugs such as cannabis, cocaine or ecstasy. By slightly modifying the chemical structures of these established drugs, NPS can be created to bypass international law enforcement regulations. The term "new" refers to substances that have recently entered the market rather than necessarily new inventions, as several NPS were first synthesized decades ago [1].

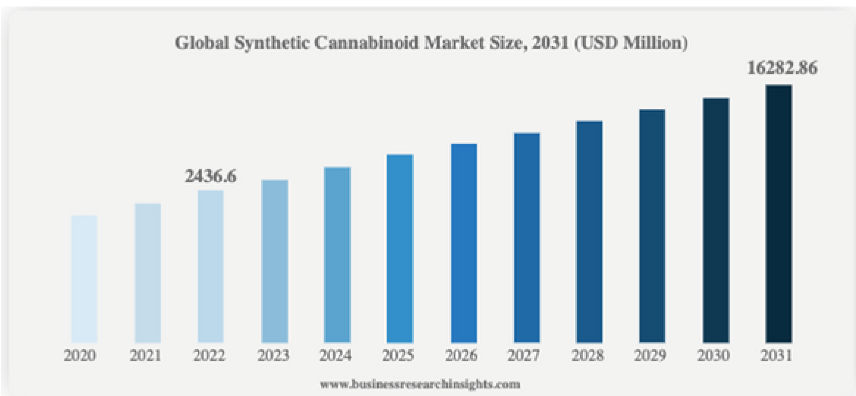
A class of chemically manufactured compounds known as synthetic cannabinoids (SCs) share chemical similarities with compounds found in the cannabis plant, but they frequently have quite different effects. By slightly modifying the chemical structures of these established drugs, NPS can be created to bypass international law enforcement regulations.

The size of the global market for SCs was estimated at 2436.6 million USDs in 2022, and by 2031, it is expected to reach 16282.86 million USDs at a forecast period compound annual growth rate (CAGR) of 23.5%. The US market generally tends to reflect the global trend of the aforementioned drug(s) (Figure 1) [2].

While many SCs are produced and sold illegally, some may have present or future medical applications [3]. Initially they were designed to provide the same effects as cannabis. However, as of recently, the newer substances brought onto the market claimed to be SCs show no resemblance to the original effects of THC (delta-9-tetrahydrocannabinol). The chemicals in these newly produced, illicitly manufactured SCs vary from batch to batch because there is no quality control. As a result, even though they are packaged the same, different packets may have different concentrations of substances and different effects.

According to a 2018 study by Cohen and Weinstein, repeated exposure to SCs causes general adverse side effects that are more severe and persistent than those associated with THC [4]. The 2019 European School Survey Project on Alcohol and Other Drugs (ESPAD) outlined that students across 20 of 35 countries reported using SCs at least once, with rates ranging from 1.1% in Slovakia to 5.2% in France [5].

Figure 1:
Size of the global market for synthetic cannabinoids (Business Research, 2024) [2].



Data on SCs use derived from population and subpopulation surveys stated use was less than 1% and in 2020 national surveys found use among 15- to 34-year-olds ranged from 0.3% in Spain and Lithuania to 0.6% in Italy [6, 7].

Long-lasting behavioural and cognitive deficits akin to those seen in rodent models of schizophrenia are brought on by repeated exposure to SCs [8]. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reports that the effects of many SCs are far stronger than THC [9] and produce more potent effects and hence increase the risks to those who consume them. Given that cannabis negatively affects cardiovascular health and given that SC drugs contain psychoactive substances that have a much higher affinity for CB1 receptors, it is not surprising that a number of reports [10] link the use of SC drugs to serious cardiovascular issues like tachycardia and myocardial infarction in both adults and adolescents [4]. Recently, there has been plenty of international deaths [9, 59] and serious poisonings due to the use or consumption of SCs.

According to the Centre for Forensic Science Research and Education (CFSRE) the prevalence and positivity of synthetic cannabinoids in the US as of 2024 (Q2 2024) was 11% (Figure 2) and research (Figure 3) shows the SCs identified [11].

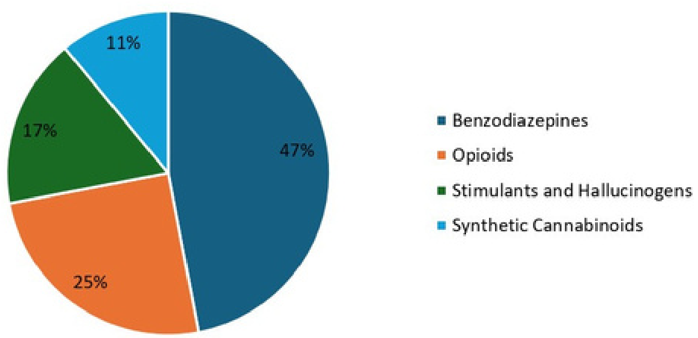


Figure 2: Q2 2024 Trend reports for SCs in the United States for the year 2024, (CFSRE, 2024) [11].

Another trend that has been on the the rise globally is the use of vaping products however these products can often contain SCs. A further challenge is the risk of developing substance abuse issues being particularly high when using vaping products for young people. It has been reported that maintaining healthy behaviour during adolescence is crucial for optimal cognitive function in adulthood [12] so there is concern as nowadays vaping behaviour is seen in much younger populations.

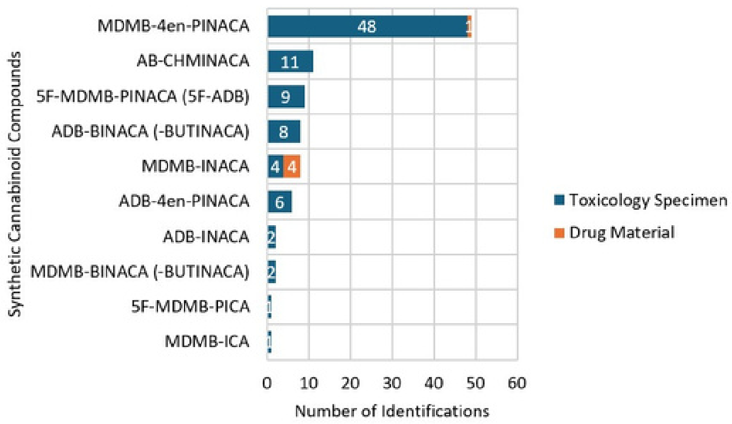


Figure 3: Synthetic cannabinoids identified in the United States in the year 2024 (CFSRE, 2024) [11].

Pharmacology:

Cannabinoid receptor agonists mimic THC and anandamide by interacting with the brain's CB1 receptor. In vitro studies show some synthetic compounds bind more strongly to this receptor than THC. The inhibitor constant (Ki) indicates an inhibitor's potency; it's the concentration needed to achieve half-maximal inhibition [13]. A lower Ki indicates a stronger binding affinity for a receptor, as the drug can occupy 50% of the receptors at a lower concentration [14].

THC has a Ki value of 10.2 nM, while other cannabinoids in smoking mixtures also have high CB1 affinity, despite some variations in reported Ki values. Another SC, HU-210, with a Ki value of 0.06 nM, binds to the CB1 receptor 100 times more tightly than THC [15].

All regions of the brain, including the cerebellum, brainstem and midbrain contain CB1 receptors. At first, it was highly probable that CB1 receptors regulated synaptic function because of their localization to neuronal terminals. It is now generally acknowledged that retrograde signalling proteins that inhibit presynaptic neurons are the primary function of CB1 receptors in the central nervous system [16].

SCs can produce effects similar to THC such as euphoria, altered perception and relaxation. However, their high potency and full agonist activity at CB1 receptors can lead to severe adverse effects [17]. In the illicit drug market, various brand names like Spice are used to sell SCs. These products typically contain unpredictable mixtures of potent synthetic CB1 receptor agonists.

The unregulated production of SCs may result in products containing harmful by-products and contaminants, heightening the risk of toxicity [18]. In summary, SCs while capable of THC-like effects, pose increased danger due to their high potency, unpredictable composition and severe adverse effects. Proper understanding and regulation are vital to mitigate associated health risks [4]. As vaping is on the rise, SCs are very prevalent in these products and new trends are being identified in terms of SCs identified.

Structural Identification:

Historically SCs fall into major structural groups: (a) Naphthylmethyl indoles, (b) Naphthoylindoles (JWH-398, JWH-073 and JWH-018), (c) Phenylacetyl indoles (i.e. Benzoyl indoles, e.g. JWK-250), (d) Naphthylmethylindenes, (e) Cyclohexyl phenols (CP 47, 497), and (f) classical cannabinoids (HU-210) [15,19].

Early warning systems are essential for identifying, tracking and mitigating the risks posed by the increasing number of SCs that are making their way into markets globally each year in a variety of products not just for vaping but also in products. Since SCs were first discovered in Europe in 2008, The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has been monitoring SCs through the European Union Early Warning System (EU EWS) [20]. Other jurisdictions have their own monitoring systems globally to address this.

Focusing on the European Union as an example the initial "official alert" of NPS being reported to the EU Early Warning System (EWS) was due to JWH-018 (shown in Figure 4) detected almost simultaneously in Austria and Germany in December 2008. These compounds generally are categorised as SCs either because of their structural similarities with other SCs or because of their pharmacological characteristics if that information is unavailable [21].

Despite their diversity, SC molecular structures can generally be classified based on common building blocks. Since 2013, the EMCDDA has focused on an approach that revolves around the main structural components: a core, a tail, a linker and a linked group to the core structure (Figure 4). Breaking SCs down structurally as demonstrated in the EMCDDA approach, offers the benefit of providing a more intricate portrayal of the molecular variations within the SC category. This is especially valuable given the recent and potential future emergence of new substances [20].

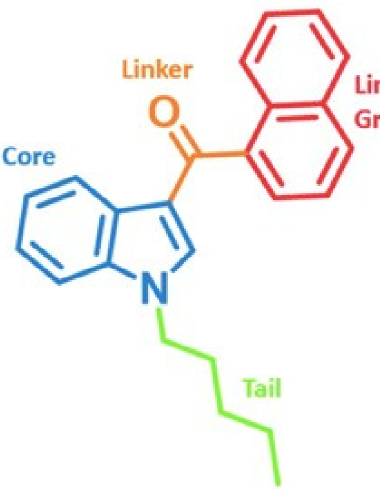


Figure 4:
The molecular structure of JWH-018, highlights the typical composition of SCs. Typical four main components: a blue core, green tail, orange linker and red linked group (Andrews et al, 2022) [20].

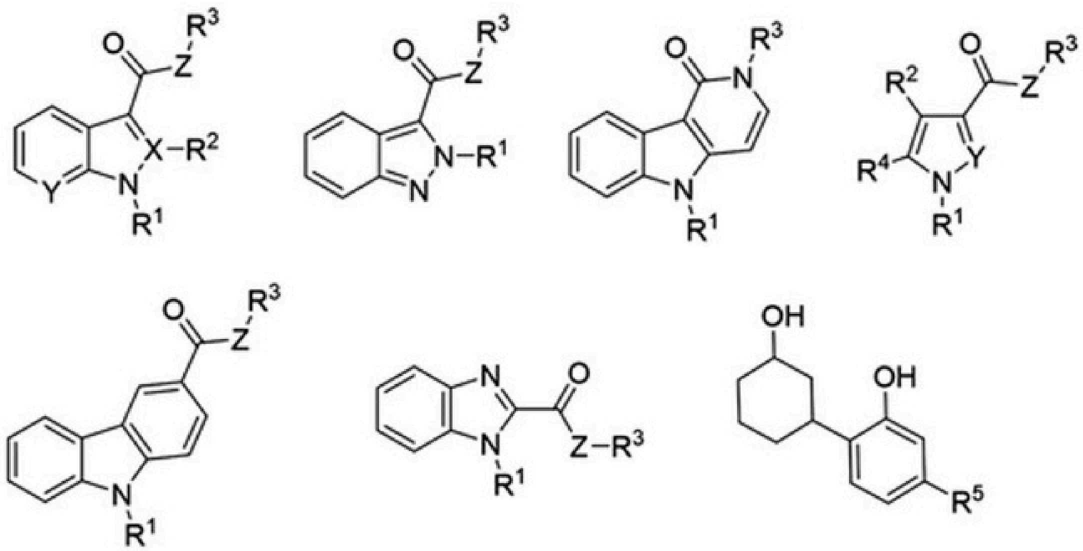


Figure 5: Core scaffold structures addressed by the 2021 Chinese legislation on SCs (Andrews et al., 2022) [24]

Outside of the European Union, Chinese legislation plays a vital role due to China's substantial contribution to the EU's SC market. Given China's influence on SC supply to Europe, its laws are likely to shape the emergence of new SCs [20]. In 2021, the Office of China National Narcotics Control Commission imposed a blanket ban on SCs, encompassing seven structural frameworks shown in Figure 5.

Since June 2021, several SCs with previously unseen or uncommon tails, cores and linkers have been identified in Europe for the first time. Notably, new compounds discovered were not covered by the generic legislation implemented in China in May 2021 [20]. The 15 monitored compounds that deviate from the existing four-part structure model were categorized as "others." These compounds included CP 47,497, CP 47,497-C6 homologue, CP 47,497-C8 homologue, CP 47,497-C9 homologue, HU-210, CP 47,497 (C8, C2), Org 27569, Org 27759, Org 29647, HU-331, trans-CP 47,497-C8, URB-754, URB-597, LY2183240, and MCHB-1 [20].

Many of these SC substances were not related structurally to the 'traditional' cannabinoids such as Δ9-tetrahydrocannabinol (THC) outlined in Figure 6. Therefore these compounds were volatile, non-polar, lipid soluble and readily smoked. A side chain was the common structural feature of these substances [15].

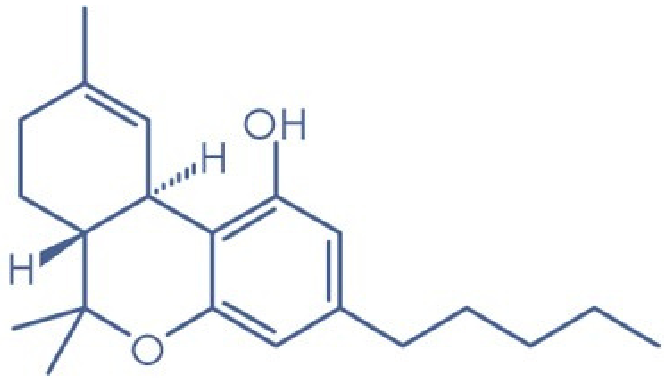


Figure 6: Delta-9 THC (European Monitoring Centre for Drugs and Drug Addiction, 2024) [15]

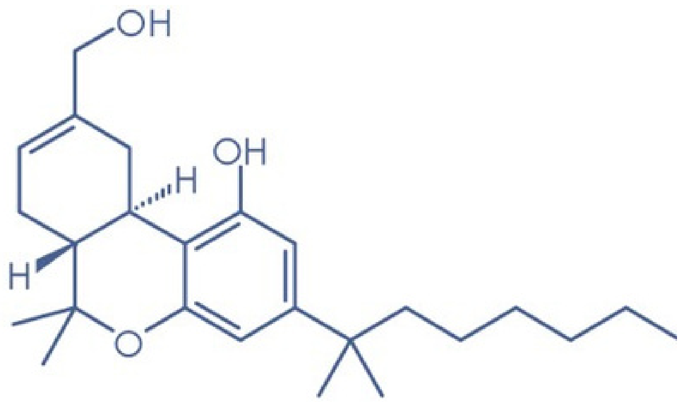


Figure 7: Molecular structure of HU-210 (European Monitoring Centre for Drugs and Drug Addiction, 2024) [15].

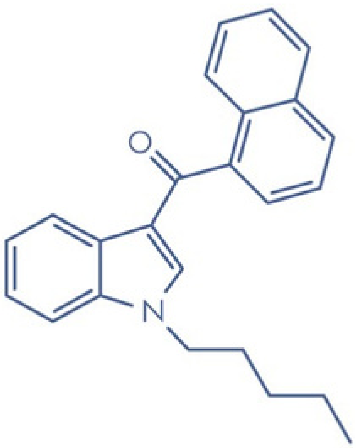


Figure 9: Molecular structure of JWH-018 (European Monitoring Centre for Drugs and Drug Addiction, 2024) [15].

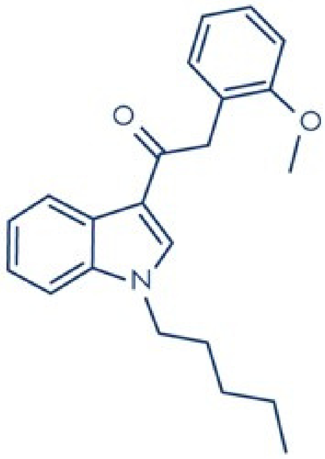


Figure 10: Molecular structure of JWH-250 (European Monitoring Centre for Drugs and Drug Addiction, 2024) [15].

Another interesting development is the rise of a multitude of products for electronic cigarettes which includes- oils, herbs or cannabis [22] but also the rise of a multitude of cannabis infused food items known as "edibles" with some edible products claiming to contain cannabis but found to contain SCs. [23,24].

In society there is a clear trend towards youth vaping so there is concern that electronic devices, cigarette oils, e-liquids and edible products may contain cannabis or other SCs. Australia became the first country to limit vape sales exclusively to pharmacies. Beginning July 1, 2024, nicotine-containing vapes will only be legally available in pharmacies to those with a doctor's prescription [25].

This is one of several measures implemented by governments to restrict the accessibility of vapes while still providing them as cessation aids for those attempting to quit smoking [26].

Overview of Δ9-THC, Δ8-THC and SCs in Products:

Another substance that is garnering a lot of attention is delta-8-tetrahydrocannabinol (Δ8-THC) as a substitute in a range of products. The main psychoactive ingredient in cannabis, delta-9-tetrahydrocannabinol (Δ9-THC), shares chemical similarities with Δ8-THC. The position of a double bond in the chemical structures is the main distinction between Δ8-THC and Δ9-THC. Δ8-THC exhibits a milder psychoactive effect and is less potent than Δ9-THC due to this slight difference [27]. The cannabis plant also contains Δ8-THC (Figure 11). It is one of more than 100 cannabinoids that the cannabis plant naturally produces though not in very large concentrations.

Δ8-THC products emerged after legalized hemp cultivation as long as THC levels remained below certain legal levels and is jurisdiction dependent. This legalization spurred companies to produce various items containing minor non-psychoactive cannabinoids like CBD and CBG along with other cannabinoids with less potent psychoactive effects than THC. These substances, derived from hemp are not considered illegal in the United States [28,29]. In 2019, Δ8-THC became available on the US market as the first synthetic cannabinoid (SC) after hemp was legalized. Δ8-THC is commonly available for purchase in a variety of forms such as vape pens, hemp leaves infused or combined with the compound and edibles such as brownies and candies [30].

There are reports of products being found with Δ8-THC and SCs. The following health problems have been reported from consuming SCs and Δ8-THC which include disorientation, breathing problems and symptoms including light headedness, a fast heartbeat, chest pain, nausea, aggression, fits, psychotic episodes, agitation, hallucinations, seizures, delusions and unconsciousness [4,31].

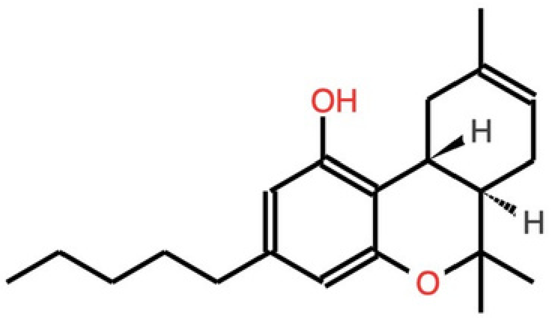


Figure 11: Δ8-THC (PubChem 2024) [32].

Globally Δ8-THC products have experienced a rapid increase in demand over the last two years, generating sales exceeding 2 billion USD as a substitute for conventional marijuana. A recent report from cannabis analytics firm Brightfield Group highlights how the growing preference for Δ8-THC products is prompting other sectors within the cannabis industry to pay attention and respond [29].

Despite international control, confusion over Δ8-THC's legal status in the United States led to a surge in related products including vaping liquids and oils and edibles products. Although hemp is legal in many jurisdictions and can be used to make Δ8-THC, its psychoactive properties make it a subject of regulatory scrutiny. While it is illegal in some states, it is legal in others under specific conditions [33].

Although Δ8-THC has been detected in Europe, its availability and application seem to be restricted. This could be because it is governed by the United Nations Convention of 1971, which requires national control throughout Europe. Furthermore, reports to the EMCDDA could potentially be under reported as Δ8-THC may occasionally go undetected [30].

Given the low natural levels of Δ8-THC in hemp, and the requirement for additional chemicals to convert other cannabinoids like CBD into Δ8-THC through synthetic means, significant concerns arise.

Manufacturers might employ unsafe household chemicals in Δ8-THC synthesis, including those used to alter the product's colour. This process may lead to harmful by-products, and there's uncertainty about additional contaminants depending on the starting material. Inhalation or ingestion of these chemicals, along with synthesis by-products poses potential health risks. Also Δ8-THC production in unregulated settings can result in unsafe contaminants or harmful substances [31]. Analysis of Δ8-THC vaping products in the United States revealed inaccurate labelling and the presence of unlisted adulterants, including by-products of chemical synthesis and heavy metals [33].

Aside from impurities such as SCs and unlabelled adulterants in vaping products, Rahman et al., 2021 found heavy metal contamination, including nickel, zinc, chromium, mercury, magnesium, copper, lead, and others in Δ8-THC vaporizers. The study reveals metal particles in unused products less than 6 months old [34]. Prior research indicated that coil heating may cause metal particles to be released. However, according to Gajdosechova et al. data indicates that at the time of purchase, these particles were already present in cannabis vape liquids. It is still unclear where these particles originated from [35].

In terms of availability, prices for smoking mixtures, enough for about eight joints, range from EUR 26 to 30 USD on internet sites or in specialty shops [15]. It is worth noting weed cart prices vary based on size, liquid type, production method, purchase method (online or in-person) and location but also supply. In research conducted by Heidelbaugh [36] in 2023, prices in shops and dispensaries were evaluated to compile estimates prices for THC (delta-9 and delta-8) and CBD carts.

The cost of a one-gram THC cart varies greatly by location due to differing tax rates and compliance costs with state regulations. Options typically range between 20 and 30 USD with many slightly above that range. High-end goods like PAX carts and premium distillates frequently cost more than 40 USD. Δ8-THC carts are currently priced similarly to CBD carts. Low-end options for a one-gram cart start at about 20 USD. Popular options usually cost 30 USD per gram, while products made with live resin typically cost between 35 and 50 USD [36]. SCs and Δ8-THC and cannabis are increasingly being found in a range of products as follows;

Vape pens or E-Liquids:

Vaping involves inhaling a mist produced by an electronic cigarette (e-cigarette). These battery-powered devices, also called vape pens, heat a liquid into an inhalable mist. The aerosol usually contains flavourings, nicotine, other potentially harmful substances and water vapor [22]. In parts of Europe, there has been an increase in vaping SCs using e-cigarettes. Sometimes, individuals unknowingly use these products believing they contain THC or CBD [37].
e-Liquids containing SCs have grown popular alongside the increased use of e-cigarettes and vaporizers. These e-liquids typically consist of a polar mixture of propylene glycol, vegetable glycerin, ethanol, aroma compounds and an active substance like SCs. The SCs used must be soluble in the liquid base, usually propylene glycol and/or glycerol with compounds like CUMYL-5F-PINACA often used [7,38].

Illicitly manufactured SCs are often added to liquid cartridges for vaping devices or mixed with dried, shredded plant material for smoking. These products are sometimes referred to as "K2" or "Spice." Users report choosing these products because they are often cheaper and more accessible than cannabis and deem them usually undetectable by routine urine drug screens [3].

While smoking cannabis remains the most popular method, e-cigarette devices for vaping cannabis-infused e-liquids are increasingly popular, especially among young adults and children.

These e-liquids often contain high concentrations of Δ9-THC, the main psychoactive ingredient in cannabis [39]. Unknown to the user, these substances can be present in cannabis (hash or weed). In the UK, they have also been found with MDMA crystals, which have resulted in hospitalizations and in 2 - CB powder, which was detected by The Loop, a drug-checking service in 2019 [40]. In June 2020, Italian Carabinieri seized e-liquid containing 5F-MDMB-PICA intended for vaping [7].

With nearly half of US states allowing legal cannabis use, the trend of vaping cannabis products is expected to continue. Additionally, e-cigarettes are being used to deliver aerosolized SCs, sometimes called "fake marijuana," "spice," or "herbal incense." The behavioural and toxic effects of synthetic cannabinoids are not well understood, but evidence links them to severe toxicities and even death [39]. According to recent samples analysed by the Welsh Emerging Drugs and Identification of Novel Substances Service (WEDiNOS), SCs are present in the majority of cannabis and THC vape products [41].

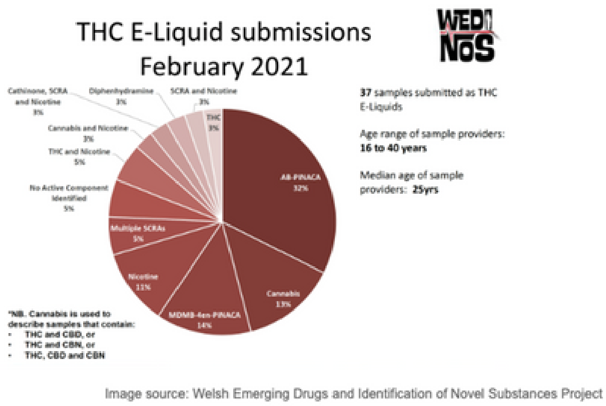


Figure 12: THC E-Liquid submissions February 2021 [41].

Food Edible Products:

Food products infused with cannabis are commonly referred to as "edibles." They come in various forms, including cakes, candies, chocolates, lozenges and sweets. These products are known by different names such as "THC Sweets," "Space Cakes," "Gummies" and "THC Lean" [42].

In recent years, cannabis-infused food items have gained popularity in the legalized markets of North America but remain prohibited in Ireland or other jurisdictions. For those who prefer not to smoke or use tobacco products, consuming cannabis through edibles is an alternative option [42]. Since edibles may have a delayed onset of effects, predicting how an individual will react becomes more challenging, increasing the risk of overconsumption or adverse reactions [43].

In 2021, synthetic cannabinoid-laced sweets were confiscated in Belgium, Estonia, Ireland, Slovakia, and Sweden often containing 5F-EDMB-PICA. Swedish customs seized 37 kilograms of such sweets in mid-2021, with the source of these products remaining unknown [37, 42]. In January 2023, Ireland issued a risk communication about SCs found in cannabis and THC products. In December 2022, a few hospitalizations were linked to the consumption of edible products in Tipperary in Ireland containing SCs [23].

Edibles pose the greatest analytical challenge as recipes and products require extensive sample preparation and cleanup techniques before analysis [44]. It's difficult to estimate THC intake and its effects due to variations in THC levels among edible products and batches. Quantitative analysis data of seized edible products in Ireland is limited, making it uncertain whether packaging accurately reflects THC content. Some seized edible products in Ireland were found to contain dangerous SCs [42]. Eating cannabis-infused edibles offers a different experience compared to smoking cannabis due to the body's reaction to ingestion.

When cannabis is eaten, the liver breaks it down into multiple psychoactive metabolites unlike smoking which bypasses this process.

While smoking converts only 20% of THC into the psychoactive compound 11-hydroxy-THC, eating converts 100% of THC into this form, resulting in potentially more potent effects [23]. Edibles don't produce immediate effects like smoking cannabis. It may take over two hours for full effects to kick in, potentially leading to overconsumption. Effects can last for several hours, with onset taking 30 to 90 minutes and peak effects not occurring for over two hours [43]. Overconsumption of edibles can lead to unpleasant or even dangerous symptoms such as paranoia, anxiety, nausea, vomiting or hallucinations [23].

Herbal mixtures:

Low-THC herbal cannabis products are increasingly accessible in certain EU countries. These products can closely resemble genuine cannabis in appearance, smell and flavour. Spraying SCs onto them is a simple and inexpensive method to enhance their potency, potentially deceiving dealers and users. Swiss police have seized production facilities and equipment used for this purpose, including one in autumn 2021 [37,41]. SCs are commonly turned into herbal mixtures for smoking and sprayed onto plant material. These products are sold under various names and brands on internet retailer sites [23]. In 2020, the EU EWS received over 6,300 seizures of SCs, comprising about 27% of all reported seizures by the EU that year. Herbal material accounted for the majority (5,100 cases, 97 kg), followed by powders (600 cases, 100 kg) [37].

Paper:

Infusing paper with SCs, like letters and photos is a common method to smuggle substances into prison. These items are then smoked, vaped or boiled to extract the substances [37]. Paper infused with SCs carries a high risk of poisoning due to significant variations in cannabinoid concentration across different parts of the paper [7].

Experiences in Ireland:

In Ireland, SC's were sold in "head shops" under different commercial brand names and 'Spice' and Black Mamba' are some examples of these.
The Criminal Justice Psychoactive Substances Act 2010 in Ireland led to the closure of "head shops" and regulated the supply of NPS substances under Irish law. Various legislative acts control NPS and other drugs in Ireland [45]. The Criminal Justice Psychoactive Substances Act 2010 is an act aimed at preventing the misuse of harmful psychoactive substances, establishing offenses for their sale, importation, exportation, advertisement and regulating the sale and advertisement of items used to cultivate certain plants in violation of the Misuse of Drugs Act 1977 [46]. Regardless of these "head shops" being forced to close, the availability of these substances in the drug market remains high [45].

Many postal packages upon analysis by Irish authorities, showed 50% of the jellies tested in 2022 contained SCs. In 2021, two preschool children were hospitalized after consuming 'jellies infused with THC [47] In December 2022, more concern arose when jellies, labelled as THC-infused, caused adverse reactions and hospitalizations of three teenagers [48]. However, analysis revealed they contained SC ADB-PINACA. In 2022, Irish authorities tested various products such as soft drinks, crisps, chocolate spread and chocolate bars and all were found to contain THC. Predicting drug marketplace trends is challenging, but the emergence of new products alongside an expanding array of SCs suggests potential future challenges in drug testing and public health [49]. Other Irish agencies outlined the usage of cannabis edibles and SCs in music festivals in Ireland [50].

The Irish Prison Service reports that in an effort to circumvent visitation restrictions prompted by COVID-19 within correctional facilities, drugs were observed being applied onto correspondence materials such as letters and newspapers (by soaking or spraying) as well as being incorporated into garments through stitching [51].

In March 2024 [52], Revenue officers in Co. Dublin, Co. Westmeath and Co. Clare seized contraband worth around €340,000 through risk profiling. Revenue officers in Co. Dublin seized 1.8 kg of cannabis edibles, 9.3 kg of herbal cannabis, 5.1 kg of mitragynine, and 72.7 g of cannabis resin, worth over €319,000. Revenue officers in Co. Westmeath seized 254.8 g of synthetic cannabinoids valued at €5,100. Illicit drugs, hidden in shipments labelled as items like clothing, pillows, bath towels and food were found in parcels from the United States, Spain the Netherlands, Indonesia and Thailand. The packages were destined for numerous counties around Ireland. Revenue officers stationed at Shannon Airport seized herbal cannabis valued at around €15,000 [52].

Conclusions:

In conclusion, the detection of cannabis, SCs and Δ8-THC in various forms including edibles, e-liquids, vaping devices, herbal mixtures, and paper, underscores a growing concern regarding their widespread availability and potential risks. Their presence in these products raises significant public health and safety concerns as consumers may unknowingly ingest or inhale substances with unpredictable effects and varying potency. As such, there is an urgent need for comprehensive regulatory measures to address the production, distribution, and consumption of these substances to mitigate associated health risks and ensure consumer safety. In 2024 Australia banned recreational vaping and were the first country in the world to do so and it will be interesting to see how this will evolve globally [25, 26].
Additionally, ongoing research and surveillance efforts are essential to monitor emerging trends and developments in the use of products containing cannabis, synthetic SCs and Δ8-THC to inform evidence-based policies and interventions aimed at protecting public health to support other countries decision making on this issue.

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Acronmyns:

MDMB-ICA: Methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1H-indazole-3-carboxamido)butanoate
5F-MDMB-PICA:Methyl 2-(1-(5-fluoropentyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate
MDMB-BINACA (BUTINACA): Methyl 2-(1-(cyclohexylmethyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate
ADB-INACA:N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide
ADB-4en-PINACA: N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-penten-1-yl)-1H-indazole-3-carboxamide
MDMB-INACA: Methyl 2-(1-(1-methyl-2-oxo-2,3-dihydro-1H-indol-3-yl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate
ADB-BINACA (BUTINACA): N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide
5F-MDMB-PINACA (5F-ADB): Methyl 2-(1-(5-fluoropentyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate
AB-CHMINACA: N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide
MDMB-4en-PINACA: Methyl 2-(1-(4-penten-1-yl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate
NPS: New Psychoactive Substances
SCs: Synthetic Cannabinoids
Delta-9 THC: Delta-9-tetrahydrocannabinol
Delta-8 THC: Delta-8-tetrahydrocannabinol
ESPAD: European School Survey Project on Alcohol and Other Drugs
EMCDDA: European Monitoring Centre for Drugs and Drug Addiction
CB1: Cannabinoid Receptor 1
CFSRE: Centre for Forensic Science Research and Education
Ki: Inhibitor Constant
CP: Cyclohexyl Phenol
CBD: Cannabidiol
CBG: Cannabigerol
e-cigarette: Electronic cigarette
MDMA: 3,4-Methylenedioxymethamphetamine
CP 47,497: 1-[(2S)-2-Hydroxy-1-(4-methoxyphenyl)butyl]-1H-indole-3-carboxylic acid
CP 47,497-C6 Homologue: 1-[(2S)-2-Hydroxy-1-(4-methoxyphenyl)hexyl]-1H-indole-3-carboxylic acid
CP 47,497-C8 Homologue: 1-[(2S)-2-Hydroxy-1-(4-methoxyphenyl)octyl]-1H-indole-3-carboxylic acid
CP 47,497-C9 Homologue: 1-[(2S)-2-Hydroxy-1-(4-methoxyphenyl)nonyl]-1H-indole-3-carboxylic acid
HU-210 : (−)-Δ9-THC (trans)-1-(2,3-dihydroxypropyl)-1H-indole-3-carboxylate
CP 47,497 (C8, C2): 1-[(2S)-2-Hydroxy-1-(4-methoxyphenyl)octyl]-2-methyl-1H-indole-3-carboxylic acid
Org 27569: 1-(2,3-dihydroxypropyl)-1H-indole-3-carboxylic acid
Org 27759: (R)-N-(2-(4-(2-hydroxyphenyl)thiazol-2-yl)phenyl)propionamide
Org 29647: 2-(2-((2,3-dihydroxypropyl)amino)-1-(4-fluorophenyl)-2-oxoethyl)-3-(4-fluorophenyl)thiazolidin-4-one
HU-331: (6aR,10aR)-2,3,4,5,6,9,10,10a-Octahydro-1H-9-oxa-6a,10a-diazapyrrolo[3,4-b]indole-1,3-dione
Trans-CP 47,497-C8: (trans)-1-[(2S)-2-Hydroxy-1-(4-methoxyphenyl)octyl]-1H-indole-3-carboxylic acid
URB-754: (S)-N-(1-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)phenyl)pyrazol-1-yl)ethyl)propanamide
URB-597: (S)-N-(2-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)phenyl)pyrazol-1-yl)ethyl)propanamide
LY2183240: N-(2-(4-(2-(4-(trifluoromethyl)phenyl)thiazol-2-yl)phenyl)thiazol-2-yl)ethyl)propanamide
MCHB-1: 1-(4-(4-(trifluoromethyl)phenyl)thiazol-2-yl)-2-(4-(trifluoromethyl)phenyl)phenyl)propenamide
JWH-018: 1-Pentyl-3-(1-naphthoyl)indole
USD: United States Dollar
CAGR: Compound Annual Growth Rate
nM: Nanomolar
THC - Tetrahydrocannabinol
e-liquids - Electronic Liquids

References:

[1] drugsandalcohol.ie [Internet]. Dublin: Health Research Board National Drugs Library; [cited 2024 February 21]. Available from: https://www.drugsandalcohol.ie/37117/.
[2] Business Research Insight [Internet]. Maharashtra: Business Research Insight; [updated 2024 May; cited 2024 February 21]. Available at: https://www.businessresearchinsights.com/market-reports/synthetic-cannabinoid-market-109498.
[3] nida.nih.gov [Internet]. National Institute on Drug Abuse Advancing Addiction Science; [cited 2024 21 February]. Available at: https://nida.nih.gov/research-topics/synthetic-cannabinoids.
[4] Cohen K and Weinstein A.M - Synthetic and Non-synthetic Cannabinoid Drugs and Their Adverse Effects-A Review From Public Health Prospective. 2018; 6: 162.
[5] : ESPAD Group (2020), ESPAD Report 2019: Results from the European School Survey Project on Alcohol and Other Drugs, EMCDDA Joint Publications, Publications Office of the European Union, Luxembourg. 2020 [cited 2024 June 02]. Available from: https://www.euda.europa.eu/publications/joint-publications/espad-report-2019_en.
[6] European Monitoring Centre for Drugs and Drug Addiction (2020), European Drug Report 2020: Trends and Developments, Publications Office of the European Union, Luxembourg. [cited 2024 June 02]. Available from: https://www.euda.europa.eu/publications/edr/trends-developments/2020_en.
[7] European Monitoring Centre for Drugs and Drug Addiction (2021), Synthetic cannabinoids in Europe – a review, Publications Office of the European Union, Luxembourg. [cited 2024 June 02]. Available from: https://www.euda.europa.eu/system/files/publications/14035/Synthetic-cannabinoids-in-Europe-EMCDDA-technical-report.pdf.
[8] Renard J, Krebs M, Jay T.M et al. Long-term cognitive impairments induced by chronic cannabinoid exposure during adolescence in rats: a strain comparison. Cerebral Cortex. 2013; 225(4):781-90.
[9] euda.europa.eu [Internet]. Portugal : European Union Drugs Agency; [cited 2024 June 02]. Available at: https://www.euda.europa.eu/spotlights/synthetic-cannabinoids_en.
[10] Barceló B, Pichini S, Coromias V. L et al. Acute intoxication caused by synthetic cannabinoids 5F-ADB and MMB-2201: A case series. 2017; 273:e10-e14.
[11] cfsre.org [Internet]. Horsham: The Centre for Forensic Science Research and Education; [cited 2024 June 02]. Available from: https://www.cfsre.org/nps-discovery/trend-reports/synthetic-cannabinoids.
[12] Publichealth.hscni.net [Internet]. Belfast: Public Health Agency; [updated 2021 April 02; cited 2024 June 02]. Available from: https://www.publichealth.hscni.net/news/spice-vaping-warning-issued-0#:~:text=The%20Public%20Health%20Agency%20(PHA,ill%20after%20vaping%20's pice'.

[13] ucl.ac.uk [Internet]. United Kingdom: London's Global University; [cited 2024 June 02]. Available from: https://www.ucl.ac.uk/~ucbcdab/enzass/inhibition.htm.
[14] simpleandpractical.com [Internet]. Simple and Practical Mental Health; [cited 2024 June 02]. Available at: https://simpleandpractical.com/ki-inhibition-constant-receptor-binding-affinity/#:~:text=In%20plain%20English%2C%20the%20Ki,occupying%2050%25%20of%20those%20receptors.
[15] euda.europa.eu [Internet]. Portugal : European Union Drugs Agency; [cited 2024 June 02]. Available from: https://www.euda.europa.eu/publications/drug-profiles/synthetic-cannabinoids_en.
[16] Mielnik C, Lam V, Ross R - CB1 allosteric modulators and their therapeutic potential in CNS disorders. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2021; 106: 110 – 163.
[17] Tai S and Fantegrossi W.E. Synthetic Cannabinoids: Pharmacology, Behavioral Effects, and Abuse Potential. Current Addiction Reports. 2014; 1(2): 129–136.
[18] Alipour A, Patel P.B, Shabbir et al. Review of the many faces of synthetic cannabinoid toxicities. Mental Health Clinician. 2019; 9(2): 93–99.
[19] redwoodtoxicology.com [Internet]. California: Redwood Toxicology Laboratory; [cited 2024 May 01]. Available from: https://www.redwoodtoxicology.com/resources/drug_info/synthetic_cannabinoids .
[20] Andrews R, Jorge R, Christie R et al. From JWH-018 to OXIZIDS: Structural evolution of synthetic cannabinoids in the European Union from 2008 to present day. Drug Testing and Analysis. 2022; 15(4): 378-387.
[21] European Monitoring Centre for Drugs and Drug Addiction (2022), European Drug Report 2022: Trends and Developments, Publications Office of the European Union, Luxembourg; [cited 2024 May 01]. Available from: https://www.euda.europa.eu/publications/edr/trends-developments/2022_en.
[22] hse.ie [Internet]. Dublin: Health Service Executive; [cited 2024 May 02]. Available from: https://www2.hse.ie/living-well/quit-smoking/vaping/#:~:text=If%20you%20have%20never%20smoked,a%20dangerous%20and%20addictive%20chemical.
[23] drugs.ie [Internet]. Drug and Alcohol Information and Support; [updated 2023 January; cited 2024 21 February]. Available from: https://drugs.ie/cannabis_edible_products/.
[24] garda.ie [Internet]. An Garda Síochána Ireland's National Police and Security Service; [cited 2024 May 01]. Available from: https://www.garda.ie/en/crime-prevention/crimecall-on-rte/crimecall-episodes/2023/22-may-2023/drugs-awareness-advice-cannabis-jellies.html#:~:text=Many%20of%20the%20sweets%20%2F%20jellies,are%20more%20potent%20than%20THC.
[25] tga.gov.au [Internet]. Australia : Australia Government. Department of Health and Aged Care. Therapeutic Goods Administration; [updated 2024 July 19; cited 2024 July 29]. Available at: https://www.tga.gov.au/products/unapproved-therapeutic-goods/vaping-hub/vapes-information-prescribers#:~:text=Vaping%20laws%20restrict%20supply%20to,pharmacies%20or%20in%20pharmacy%20settings.
[26] Ibrahim T. abc.net.au [Internet]. Australian Broadcasting Corporation ; [cited 2024 July 30]. Available from: https://www.abc.net.au/news/2024-06-25/what-you-need-to-know-about-australia-move-to-ban-vape/104016192 .
[27] Kapur B and Aleksa K - What the lab can and cannot do: clinical interpretation of drug testing results. Critical Reviews in Clinical Laboratory Sciences. 2019; 57 (8): 548-585.
[28] Sabaghi D. forbes.com [Internet]. Forbes ; [cited 2024 June 04]. Available from: https://www.forbes.com/sites/dariosabaghi/2023/01/16/delta-8-thc-generated-2-billion-in-revenue-in-2-years-report-finds/.
[29] cannabisbusiness.com [Internet]. Skodzinski N: Cannabis Business Times; [cited 2024 June 04]. Available from: https://www.cannabisbusinesstimes.com/news/how-big-is-us-market-for-delta-8-thc-and-hemp-derived-cannabinoids/#:~:text=The%20U.S.%20market%20for%20Delta%2D8%20THC%20and%20other%20hemp,and%20analytics%20firm%20Brightfield%20Group.
[30] euda.europa.eu [Internet]. Portugal: European Monitoring Centre for Drugs and Drug Addiction; [cited 2024 23 March]. Available from: https://www.euda.europa.eu/publications/technical-reports/hhc-and-related-substances_en.
[31] fda.gov [internet]. Food and Drugs Administration; [updated 2022 April 05; cited 2024 June 02]. Available from: https://www.fda.gov/consumers.
[32] National Center for Biotechnology Information [Internet]. PubChem Compound Summary for CID 638026, DELTA8-Tetrahydrocannabinol; [cited 2024 June 05]. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/638026.
[33] euda.europa.eu [Internet]. Portugal :European Monitoring Center for Drugs and Drug Addiction; [cited 2024 May 31]. Available from: https://www.euda.europa.eu/publications/eu-drug-markets/cannabis_en.
[34] Atrash J.M and Rahman I - Novel Δ8-tetrahydrocannabinol vaporizers contain unlabeled adulterants, unintended byproducts of chemical synthesis, and heavy metals. Chemical Research in Toxicology. 2022; 35(1): 73-76.
[35] Gajdosechova Z, Gillette J.M, Turnbull M et al. Evidence That Metal Particles in Cannabis Vape Liquids Limit Measurement Reproducibility. ACS Omega. 2022; 7(47): 42783–42792.
[36] vaping360.com [Internet]. Heidelbaugh H: Vaping 360; [updated 2023 September 08; cited 2024 June 02]. Available from: https://vaping360.com/learn/how-much-is-a-cart/.
[37] European Monitoring Centre for Drugs and Drug Addiction (2022), New psychoactive substances: 25 years of early warning and response in Europe. An update from the EU Early Warning System (June 2022), Publications Office of the European Union, Luxembourg; [cited 2024 June 01]. Available from: https://www.euda.europa.eu/publications/rapid-communication/update-eu-early-warning-system-2022_en.
[38] Angerer V, Franz F, Moosmann B et al. 5F-Cumyl-PINACA in 'e-liquids' for electronic cigarettes: comprehensive characterization of a new type of synthetic cannabinoid in a trendy product including investigations on the in vitro and in vivo phase I metabolism of 5F-Cumyl-PINACA and its non-fluorinated analog Cumyl-PINACA. 2019; 37(1): 186–196.
[39] Timothy W Lefever, Thomas B.F, Marusich J.A et al. Vaping Synthetic Cannabinoids: A Novel Preclinical Model of E-Cigarette Use in Mice. Substance Use: Research and Treatment 11. 2017; 11 (!).
[40] drugs.ie [Internet]. Drug and Alcohol Information and Support; [cited 2024 June 02]. Available from: https://www.drugs.ie/drugtypes/drug/headshop_drugs_smoke_synthetic_cannabinoids.
[41] frugs.ie [Internet]. Drug and Alcohol information and Support; [cited 2024 June 02]. Available from: https://www.drugs.ie/update_may_2021_synthetic_cannabinoids/.
[42] drugs.ie [Internet]. Drug and Alcohol Information and Support; [updated 2023 January; cited 2024 May 31]. Available from: https://drugs.ie/cannabis_edible_products/.
[43] Drugs.ie [Internet]. Drug and Alcohol Information and Support; [cited 2024 May 31]. Available from: https://www.drugs.ie/resources/factsheets/.
[44] Leghissa A, Hildenbrand Z.L, Schug K. A et al. The imperatives and challenges of analyzing Cannabis edibles. Current Opinion in Food Science. 2019; 28: 18-24.
[45] Drug.ie [Internet]. Drugs and Alcohol Information and Support - About New Psychoactive Substances; [cited 2024 June 03]. Available from: https://www.drugs.ie/drugtypes/drug/aboutnps.
[46] Irishstatute.ie [Internet]. The Criminal Justice (Psychoactive Substances) Act 2010; [cited 2024 June 03]. Available from: https://www.irishstatutebook.ie/eli/2010/act/22#:~:text=AN%20ACT%20TO%20PREVENT%20THE,IN%20THE%20CULTIVATION%20OF%20CERTAIN
[47] thejournal.ie [Internet]. Journal Media Ltd; [updated 2021 September 20; cited 2024 July 29]. Available from: https://www.thejournal.ie/edible-cannabis-children-dublin-5553817-Sep2021/.
[48] irishexaminer.com [Internet]. Examiner Echo Group Limited; [updated 2023 January 06; cited 2024 July 29]. Available from: https://www.irishexaminer.com/news/munster/arid-41042564.html.
[49] forensicscience.ie [Internet]. Dublin: Forensic Science Ireland; [cited 2024 June 04]. Available from: https://forensicscience.ie/documents/FSI%202022%20Annual%20Report.pdf.
[50] rte.ie [Internet]. Raidió Teilifís Éireann (RTÉ); [updated 2023 May 22; cited 2024 June 04]. Available from: https://www.rte.ie/news/ireland/2023/0522/1384942-drug-testing-festivals/#:~:text=A%20drug%20testing%20regime%2C%20introduced,Festival%20in%20Mullingar%20this%20weekend.
[51] irishexaminer.ie [Internet]. Examiner Echo Group Limited; [updated 2023 January 29; cited 2024 June 04]. Available from: https://www.irishexaminer.com/news/arid-41059052.html.
[52] revenue.ie [Internet]. Revenue Irish Tax and Customs; [updated 2024 March 22; cited 2024 June 04]. Available from: https://www.revenue.ie/en/corporate/press-office/press-releases/2024/pr-032224-contraband.aspx#:~:text=office%20Press%20releases-,Revenue%20seize%20contraband%20worth%20almost%20%E2%82%AC340%2C000%20while%20examining%20parcels,a%20result%20of%20risk%20profiling.
[53] adf.org.au [Internet]. Alcohol and Drug foundation; 2024 [updated 2024 June 06; cited 2024 February 2024]. Available from: https://adf.org.au/drug-facts/new-psychoactive-substances/.
[54] drug.ie [Internet]. Drug and Alcohol Information and Support; [cited 2024 June 04]. Available from: https://www.drugs.ie/synthetic_cannabinoids_in_ireland_hse_update_october_2021/.
[55] rte.ie [Internet].]. Raidió Teilifís Éireann (RTÉ); [cited 2024 June 04]. Available from : https://www.rte.ie/news/health/2024/0105/1424749-drugs-synthetic-hse/.

Career Spotlight:
Professor Ashraf Mozayani

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Current Practice Areas/Industries:

I am a researcher and practitioner in the field of forensic science with expertise in various areas. I have worked on topics such as postmortem forensic toxicology, impaired driving and drug-facilitated crimes particularly focusing on the use of alcohol and drugs in sexual assaults. My main expertise lies in understanding how drugs work in postmortem cases and their impact on human behavior.
Additionally, I am involved nationally and globally in improving forensic science by setting high standards for laboratories and leadership in the forensic field. My research reflects my commitment to advancing knowledge, promoting criminal justice reform and contributing to a better society through scientific excellence in the ever-changing world of forensic science.

Education:
I have a Doctor of Pharmacy degree from the University of Tehran, as well as a PhD from the University of Alberta in Pharmaceutical Science and Toxicology. I am also a board-certified fellow of the American Board of Forensic Toxicology.

Did you always hope to work in toxicology? What did you do in school to prepare yourself for those opportunities:

While studying for my Pharm.D., I held a part-time job in a hospital lab. After obtaining my Pharm.D., I worked as Pharmacist and directed my own community pharmacy in Iran for more than seven years. After immigrating to Alberta, Canada, I changed career paths and chose not to get my license in pharmacy but instead pursue a PhD in Pharmaceutical Science and Toxicology.

However, my journey into toxicology took a turn when I enrolled in a course on forensic toxicology taught by the renowned Dr. Graham Jones.

Dr. Jones offered his students an opportunity to visit his laboratory at the office of the Alberta Medical Examiner and that visit completely transformed my perspective. The experience of touring his lab introduced me to an entirely new field that I had never considered before. Interestingly, fate seemed to have a role in my transition into toxicology. One of my PhD colleagues happened to come across a job opening at the office of the medical examiner and shared it with me. I decided to apply for the position and was fortunate enough to secure an interview.

This was a significant shift for me as I had never before applied for a job in North America. In preparation for the interview, I spent three days constantly studying every available resource on interviewing techniques.

To my surprise, Dr. Jones showed a keen interest in my previous experience in Iran, where I had worked in a clinical laboratory while pursuing my Pharm.D. degree. It was a side job during my university years and I had never imagined that it would play a pivotal role in landing my first job in this field.

Prior to my move to the United States and my exposure to the field of forensic toxicology, I had no idea about Dr. Jones's status as a well-known figure in this domain. This unexpected journey was a remarkable and enriching experience, leading me into a career that I had never anticipated.

What work did you do following your initial undergraduate and postgraduate training if applicable trainings undertaken:

I started my job in the Toxicology Lab at the Chief Medical Examiner's Office in Calgary while working on my thesis.

What do/did you most enjoy about your work:

I find immense satisfaction in delving into intricate and varied challenges of the forensic sciences, particularly expanding research in the subjects of forensic toxicology, criminal justice reform and leadership. The intellectual challenge of deciphering convoluted cases in both criminal and civil domains and illuminating issues like impaired driving, drug-facilitated crimes and postmortem examinations brings me a sense of purpose. Additionally, I take great pride in my role in enhancing global standards for high-quality laboratory practices and court testimonies. In essence, my work not only allows me to advance knowledge but also enables me to drive reform and make a positive impact on my students and society, all through the pursuit of scientific excellence.

What might you do differently now that you have had all the experiences you have had if you had the chance to do it again:

Spend more time with my children, extended family and friends.

What do you like to do in your free time:

Spend quality time with my family and friends, especially my grandchildren. I cherish the time I spend with them, whether at home or traveling.

Hallucinogenic Mushrooms and Psilocybin

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Keywords: Psilocybin, Hallucinogenic Mushrooms, Psychedelic Mushrooms, Substance Use Disorders

Hallucinogenic mushrooms, also known as psilocybin mushrooms, are a group of fungi that contain the compound psilocybin. They have been consumed by numerous cultures for many centuries. The Aztecs called them "God's flesh" and ingested them during religious and healing ceremonies [1]. Mushroom specimens were first brought into the Western world in the 1930s and the main psychedelic compound, psilocybin, was first isolated and identified in the late 1950s by Albert Hofmann. Since then, psilocybin mushrooms have been classified as a schedule I drug in the United States of America, and banned in most other countries, with warnings about their potential dangers [2]. However, several scientific studies and clinical trials have reported a very low toxicity and abuse potential for psilocybin, especially when compared to common substances of abuse such as opioids, ethanol and tobacco. Additionally, several studies have investigated the potential therapeutic uses of psilocybin for conditions such as substance use disorders (SUDs) and depression and their results suggest that psilocybin may be beneficial for people diagnosed with these conditions by improving their emotional states and reducing stress and anxiety [3].

There are currently approximately 300 species of hallucinogenic or psychedelic mushrooms that are described in the literature. Given the growing interest in the medicinal use of psilocybin in recent years, it is expected that more species will be discovered and researched in the future. Hallucinogenic mushrooms are divided into several genera such as P*silocybe*, *Panaeolus*, *Pluteus, Gymnopilus, Inocyb*e and *Pholiotina* but most of the pharmacologically-important species belong to the genus *Psilocybe* [4].

Evolutionarily, the presence of psilocybin in these fungi have been associated with protection from mycophagous insects by affecting their behaviour [5]. Morphologically, the mushrooms are small and have white-brown colours and more importantly, they form blue bruises when damaged or cut. This blue colouring is the result of an oxidation reaction which occurs when psilocybin is exposed to oxygen in the air [4]. These physical characteristics often prove important in the identification of these types of mushrooms by law enforcement and medical researchers. The concentration of psilocybin contained in the various types of mushrooms has been measured with high-performance liquid chromatography and several other techniques [6,7].

Figure 1. The blue colouration of damaged P*silocybe cubensis* specimen (right) compared to intact specimen (left) [5].

Due to their bitter taste, psilocybin mushrooms are usually dried and ground into powder which can then be added to sweet edible treats [8]. Due to this preparatory process, mushrooms that are seized during police investigations are usually not possible to be identified based on their morphological characteristics since none are visible in powders or sweets. In these cases, a DNA-based approach may be required in order to determine the species of psilocybin mushroom [9]. Indeed, DNA-analysis was used to identify samples seized from an illicit laboratory that was cultivating mushrooms in Chile in 2018. With the use of genetic analysis and electron micrograph imaging, the mushrooms were identified to be hallucinogenic *Psilocybe cubensis* [10]. Besides law enforcement reasons, correct species identification of hallucinogenic mushrooms may also be important to avoid accidental ingestion of psilocybin. For instance, the edible honey fungus, *Armillaria mellea*, morphologically looks very similar to the hallucinogenic *Gymnopilus spectabilis* resulting in instances where *G. spectabilis* was accidentally ingested in place of *A. mellea* [4].

As a serotonergic compound, psilocybin is structurally very similar to the neurotransmitter serotonin and interacts with serotonin receptors in the brain [8]. The effects of psilocybin are mainly mediated through the activation of serotonin receptors, also called 5-hydroxytryptamine (5-HT) receptors, located in several parts of the brain. Psilocybin's interaction with 5-HT2A receptors is essential for its hallucinogenic effects. After oral ingestion of hallucinogenic mushrooms, psilocybin is rapidly converted into the lipid-soluble psilocin via dephosphorylation [3]. Psilocin easily crosses the blood-brain barrier and undergoes further metabolism by demethylation and oxidative deamination catalysed by several different enzyme systems including monoamine oxidase (MAO). The MAO involved step seems to be important for the duration of the hallucinogenic effects of psilocybin when psilocybin mushrooms are taken alongside MAO-inhibitors such as phenelzine [11] and the metabolism of psilocin is delayed which consequently prolongs the intensity and duration of the hallucinogenic effects for the user. A similar effect is observed when alcohol is concurrently consumed with psilocybin mushrooms since the primary metabolite of ethanol, acetaldehyde, reacts with endogenous biogenic amines to produce MAO-inhibitors such as β-carbolines [8]. This suggests that the MAO pathway can be manipulated by the mushroom users to prolong and enhance their hallucinogenic "trip".

Substance use disorders (SUDs) are, according to the World Health Organisation (WHO), a significant issue with which 35 million people struggle worldwide [12]. Traditionally, both psychological and pharmacological interventions are involved in the treatment of SUDs. Despite the increasing effectiveness of these treatments, patients struggling with SUDs have a relatively high relapse rate: approximately 60% within 6-12 months after the treatment [13]. Psilocybin may be a suitable treatment for SUDs as several studies have shown that it may improve negative emotional states, reduce stress and decrease the likelihood of subsequent substance use [3,14-17].

The therapeutic potential of psilocybin is thought to be associated with the neuroanatomical distribution of 5-HT receptors in the brain [3]. There is a high density of 5-HT2A receptors both in the brain's amygdala which is responsible for emotional learning and in the ventral striatum which regulates the reward system. The amygdala and the striatum are thought to play important roles in the formation of SUDs through their involvement in the formation of cue-induced drug seeking behaviours, negative emotional states and negative reinforcement [3]. This was demonstrated in a functional magnetic resonance imaging (fMRI) study where acute psilocybin administration caused a decrease in amygdala reactivity which was associated with an improvement in emotional state and an increase in positive thinking in subjects [17].

A 2022 randomised clinical trial investigated the effect of psilocybin on alcohol-dependence (considered a SUD) and involved participants who were diagnosed with alcohol-dependence [15].

Participants randomly received either psilocybin or a placebo during the study alongside psychotherapy provided by a group of licensed therapists and psychiatrists. The results showed that the percentage of heavy drinking days for the psilocybin-administered group was 41% of that of the placebo group which suggests that heavy drinking was significantly lower in the psilocybin-treated group compared to the placebo. The participants who received psilocybin were also much more likely to have no drinking days and had significant reductions in drinking-related issues [15]. The findings of this study suggest that psilocybin may be an effective treatment for alcohol-dependence, especially combined with psychotherapy.

Several clinical and toxicological studies indicate a very low toxicity and abuse potential for psilocybin [2,18-20]. Additionally, in both rats and pigs, there was no evidence of toxicity to isolated organs [21]. LD50 of psilocybin for rats was found to be 280 mg/Kg which is a very high dose that is not practically achievable by ingesting psilocybin mushrooms. Assuming that the LD50 in rats could apply to humans, a 60-Kg human would need to ingest approximately 1.7 Kg of fresh psilocybin mushrooms to reach such a high dose of psilocybin [8]. Several authors, however, have reported adverse side effects caused by psilocybin. The most common adverse effects were confusion, dysphoria, anxiety, headaches and nausea which were experienced by only a small proportion of participants [2,22]. Indeed, a pooled analysis of experimental studies published in 2011 reported that only 7% of participants who received a high dose of psilocybin experienced adverse psychotic reactions. The same analysis also reported that the adverse effects were short-term and went away within 24 hours of psilocybin administration [22].

The authors also reported that there was no reported increase in subsequent abuse of psilocybin or of other substances after participation in the study which suggests that psilocybin does not induce drug-seeking behaviours or withdrawal symptoms [22].

Additionally, a study on rhesus monkeys found that the animals did not self-administer psilocybin suggesting it didn't produce addictive effects [23]. It has been suggested that the low abuse potential of psilocybin may be explained by the fact that it does not directly interact with the mesolimbic dopaminergic system and thus does not activate the reward system of the brain [2]. Interestingly, a number of participants in psilocybin studies have described their experiences with psilocybin as “tiring” and reported that they welcomed the “coming down” from the hallucinogenic effects, even if they had a pleasant “trip” [22].

The principal psychoactive compound in hallucinogenic mushrooms is the prodrug psilocybin which is converted to psilocin in the body. Different mushroom species contain differing amounts of psilocybin and may therefore exert varying degrees of hallucinogenic effects on the user. According to a number of clinical studies, psilocybin has low toxicity and abuse potential, especially when compared to substances like alcohol, tobacco and opioids. As described above, there exist several different genera of hallucinogenic mushrooms that are pharmacologically important. Until now, their identification stemmed from law-enforcement and public safety concerns but it is now rapidly recognised that medicinal and pharmacological benefits may exist which mandate further research. Psilocybin appears to have great potential in a therapeutic setting, particularly in the treatment of conditions such as substance use disorders. Several studies have reported beneficial effects of psilocybin on increasing positive moods and reducing stress and anxiety on patients struggling with SUDs. This finding mandates more research including clinical trials to evaluate its therapeutic potential as well as the possible interactions of psilocybin with other drugs and treatment types.

Acronymns:

Substance use disorders (SUDs)
5-hydroxytryptamine (5-HT)
Monoamine oxidase (MAO)
Lethal dose 50 (LD50)

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References:

[1] Nichols DE. Psilocybin: from ancient magic to modern medicine. The Journal of Antibiotics. 2020;73(10):679–686.

[2] Tylš F, Páleníček T, Horáček J. Psilocybin – Summary of knowledge and new perspectives. European Neuropsychopharmacology. 2014;24(3):342–356.

[3] de Veen BTH, Schellekens AFA, Verheij MMM, et al. Psilocybin for Treating Substance Use disorders? Expert Review of Neurotherapeutics. 2016;17(2):203–212.

[4] Strauss D, Ghosh S, Murray Z, et al. An Overview on the Taxonomy, Phylogenetics and Ecology of the Psychedelic Genera Psilocybe, Panaeolus, Pluteus and Gymnopilus. Frontiers in Forests and Global Change. 2022;5.

[5] Lenz C, Wick J, Braga D, et al. Injury-Triggered Blueing Reactions of Psilocybe 'Magic' Mushrooms. Angewandte Chemie International Edition. 2020;59(4):1450–1454.

[6] Bigwood J, Beug MW. Variation of psilocybin and psilocin levels with repeated flushes (harvests) of mature sporocarps of Psilocybe cubensis (earle) singer. Journal of Ethnopharmacology. 1982;5(3):287–291.

[7] Goff R, Smith M, Shams Ul Islam, et al. Determination of psilocybin and psilocin content in multiple Psilocybe cubensis mushroom strains using liquid chromatography – Tandem mass spectrometry. Analytica Chimica Acta. 2024;1288:342161-342161

[8] Dinis-Oliveira RJ. Metabolism of psilocybin and psilocin: clinical and forensic toxicological relevance. Drug metabolism reviews. 2017;49(1):84–91.

[9] Nugent KG, Saville BJ. Forensic analysis of hallucinogenic fungi: a DNA-based approach. Forensic Science International. 2004;140(2–3):147–157.

[10] Solano J, Anabalón L, Figueroa S. Psychedelic fungus (Psilocybe sp.) authentication in a case of illegal drug traffic: sporological, molecular analysis and identification of the psychoactive substance. Science & Justice. 2019;59(1):102–108.

[11] Halman A, Kong G, Sarris J, et al. Drug–drug interactions involving classic psychedelics: A systematic review. Journal of Psychopharmacology.2024;38:3-18

[12] World Health Organization. Global Status Report on Alcohol and Health and Treatment of Substance Use Disorders. 2024. Accessed 270724

[13] Cornelius JR, Maisto SA, Pollock NK, Martin CS, Salloum IM, Lynch KG, Clark DB. Rapid relapse generally follows treatment for substance use disorders among adolescents. Addictive Behaviors. 2023;28(2):381–386.

[14] Bogenschutz MP, Forcehimes AA, Pommy JA, Wilcox CE, Barbosa PCR, Strassman RJ, Psilocybin-assisted treatment for alcohol dependence: a proof-of-concept study. Journal of psychopharmacology (Oxford, England). 2015;29(3):289–99.

[15] Bogenschutz MP, Ross S, Bhatt S, et al. Percentage of heavy drinking days following psilocybin-assisted psychotherapy vs placebo in the treatment of adult patients with alcohol use disorder. JAMA Psychiatry. 2022;79(10).

[16] Kometer M, Schmidt A, Bachmann R, et al. Psilocybin biases facial recognition, goal-directed behavior, and mood state toward positive relative to negative emotions through different serotonergic subreceptors. Biological psychiatry. 2012;72(11):898–906.

[17] Kraehenmann R, Preller KH, Scheidegger M, et al. Psilocybin-Induced Decrease in Amygdala Reactivity Correlates with Enhanced Positive Mood in Healthy Volunteers. Biological Psychiatry. 2015;78(8):572–581.

[18] Johnson MW, Griffiths RR, Hendricks PS, et al. The abuse potential of medical psilocybin according to the 8 factors of the Controlled Substances Act. Neuropharmacology. 2018;142(142):143–166.

[19] Passie T, Seifert J, Schneider U, et al. The pharmacology of psilocybin. Addiction Biology. 2002;7(4):357–364.

[20] Roscoe J, Lozy O. Can psilocybin be safely administered under medical supervision? A systematic review of adverse event reporting in clinical trials. Drug Science, Policy and Law. 2022;8:205032452210852.

[21] Cerletti A. Etude pharmacologique de la psilocybine. In Heim, R., Wasson, R.G. (Eds.), 1st ed. Editions du MuseumNational d'Historie Naturalle 1958;268–271.

[22] Studerus E, Kometer M, Hasler F, et al. Acute, subacute and long-term subjective effects of psilocybin in healthy humans: a pooled analysis of experimental studies. Journal of Psychopharmacology. 2011;25(11):1434–1452.

[23] Fantegrossi WE, Woods JH, Winger G. Transient reinforcing effects of phenylisopropylamine and indolealkylamine hallucinogens in rhesus monkeys. Behavioural Pharmacology. 2004;15(2):149–157.

Toxicology: a strange discussion between ''Pharmacos'' and ''primum non nocere''

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Keywords: Drugs of Abuse, Impaired Driving, Pharmacos, Disease, Xenobiotics, Toxicology, Odyssey

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Toxicology is a science that tells stories which change lives. For example, a 24-years-old young man, driving erratically, stopped by the police at around 11:00 pm. As he presented symptoms of intoxication, he was taken to the hospital for a blood and urine sample to be analysed. Immunochemical tests showed the presence of benzodiazepines and cocaine. GC-MS confirmed the presence of cocaine, phenacetin, diazepam, fluoxetine and caffeine. The GC-MS and LC-MS quantification showed cocaine (27 μg/L), benzoylecgonine (1,350 μg/L), ethylcocaine (29 μg/L), fluoxetine (370 μg/L), diazepam (880 μg/L) and ethanol (0.99 μg/Kg). These results explain the impairment of the driver.
This story is more than a simple case [1] of road "delinquency"; it resonates as a symptom of a stray society. It shows a 24-year-old boy using an illegal drug such as cocaine, a social drug such as alcohol and doctor-prescribed medications such as Valium® and Fluctine®. This case raises the question of the simultaneous use of different xenobiotics. Today, there is much debate about the liberalization of drugs, but few debates about medication abuse. Perhaps we need to go back in history to understand the origin of the theory about drugs and medicine.

In ancient Greece, the word "pharmacos" hid three elements of information: firstly, the concept of the scapegoat, i.e. the person who, through his sacrifice, solved all the problems of a sick city. But the word also covers the notion of poison and medicine. It was probably thought that to resolve suffering, a molecule had to handle the body's suffering, either by curing it or killing it. The use of antidepressants, benzodiazepines, alcohol and cocaine would simply be the scapegoats for that living city this young man has built. In this context then xenobiotics become the solution that extinguishes the symptoms and raises the question of the difference between a drug and a medicine.

In ancient Greece, Hippocrates, (considered as the father of medicine) built this art of service to humanity on a cornerstone: "primum non nocere". This medicine treated the cause of illness, as revealed by the symptoms. Our modern medicine, built with guidelines, supports numerous therapies using xenobiotics, which are found in many cases of people tested in drivers on our roads and particularly in the case of our young 24-year-old driver. This raises questions about prescribing in modern medicine.

Statistics from the Swiss Federal Office of Public Health (FOPH) [2] show that, in 1992, 38% of people over the age of 15 in Switzerland were taking drugs; by 2022, this figure rose to 55%. Thus, in Switzerland in 2022, one person in two will be taking medication (painkillers, sleeping pills, benzodiazepines, antidepressants, etc.). Among the over-75's, this proportion is as high as 84%. This high level of consumption means that it is highly likely that many xenobiotics will be detected on the road in the case of police control.

Toxicology is a science used by medicine, the police and the justice system for many years in an attempt to understand human behavior in madness, anger, crime and addiction. To answer these questions, it needs increasingly sophisticated techniques such as GC-MS and/or LC-HRMS. So, even in trace amounts, many substances can be detected, considering of course all the restrictions involved in drug metabolism.

But when the assay is complete, the quality controls validated, the drugs detected and the report signed, can we solve the questions that emerge in the life story of the drivers and of this driver in particular? Probably not.

Toxicology can only identify the "pharmacos" without understanding the cause of their consumption. Would the results of toxicology analyses reveal another, more fundamental question summarized in these word: ‘’quo vadis’’? In other words, what is life?

Life is a strange journey, perhaps an odyssey. Indeed, this earthly time is made up of different seasons. First, the period that takes us towards battlefield, those where we have to fight to become someone in this society, someone in the eyes of others. It is the period of our studies, our training and our professional life. In this theater scene we play the role of our lives. Then, as time passes, another era comes along, like a return from war, a journey towards ourselves that would allow us to become someone in our own eyes. This return is sometimes stimulated by a change, a retirement, a new profession, an encounter, a police control or even sometimes an illness that finally leads us to ask ourselves a question we've heard or read about but due to our materialism or ourselves. we hide deep down inside or forget the question of "who am I?" Therefore, it seems there is always a moment in our journey that prompts us to rework the "Nosce te ipsum".

Moreover, like Ulysses, we have to discover that life may be a trip in a world built in three dimensions. Firstly [3], there is our body, which is the perishable dimension that medicine treats as best it can by applying the "primum non nocere". Second our soul, which is the psychological dimension that motivates our desires. Finally, our spirit that allows us to see an eternal light. As believed by the ancients, health was the fruit of a happy association between these three dimensions and that illness, which the body expresses in various symptoms, is born of their miscommunication when we do not see the light anymore.

Therefore, between medicine, which tries to understand and toxicology which detects, perhaps we need to work on a third dimension, a psycho-spiritual approach in which we will find the path of the whys and wherefores of our consumption.

Acronymns:

Gas Chromatography Mass Spectrometry (GC-MS),
Liquid Chromatography- High Resolution Mass spectrometry (LC-HR-MS), Liquid Chromatography–Mass spectrometry (LC-MS)

References:

[1] Joye T, Déglon J, Donzé N. et al. Randomly Controlled Drivers Using Minimally Invasive Sampling: Assessment of Drug Prevalence in Western Switzerland over Two Time Periods. BMC Public Health 2022, 2446

[2] Enquête Suisse sur la Santé 2022: Tableaux Standard La Consommation de Médicaments en Hausse Depuis 30 ans (OFSP) Accessed 22/06/24

[3] Von Bingen H, Hozeski BW,Hildegard's Healing Plants: From Her Medieval Classic Physica, Beacon Press, 2002, May 11

Enantioseparation of three synthetic cathinones: an evaluation of CHIRALPAK® AD-3 column using High Performance Liquid Chromatography

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Over the preceding decades, studies of chiral compounds have been growing in pharmaceutical, toxicological, food, environmental and other analytical approaches due to different pharmaceutical activities of the enantiomers. It is well known that one of the enantiomers is biologically active, whereas the other may be inactive or pose undesired effect [1]. The action of chiral compounds in living organisms may involve stereoselective absorption, distribution, metabolism and excretion phenomena [2]. The fate of chiral drugs in biological systems can be studied by monitoring their enantiomeric ratio during various biological processes. This information may be used to design and develop safe chiral drug medications [3]. For example, thalidomide was first marketed as racemate before it was later discovered that the teratogenic effects were mainly produced by the *S*-enantiomer of thalidomide [4, 5].

Analytical methods are therefore required to discriminate between the two enantiomers of a new racemic drug to avoid such scenarios. All new designer drugs derived from cathinone, the principal active ingredient present in the leaves of the *Catha edulis* (Khat) plant, have a chiral center at their α-carbon yielding two stereoisomers [6]. Owing to the novelty of these compounds, limited data about the pharmacological and toxicological, and consequently about the single enantiomers are available. It was found that (*S*)-enantiomer of methcathinone poses more psychoactive effects than their (*R*)-antipode [7]. This is unsurprising as methcathinone is an analog of cathinone where most of its toxicological effects were attributed to the (–)-enantiomer [8,9]. Various analytical techniques dealing with the chiral separation of cathinone derivatives can be found in the literature [1, 10–15]. Among them, liquid chromatography, especially high-performance liquid chromatography (HPLC), has achieved a successful separation of cathinone enantiomers [16].

In particular, the direct separation of enantiomers by chiral stationary phases (CSPs). Diverse CSPs used in HPLC columns namely polysaccharide, cyclodextrin, proteins, antibiotics, crown ethers and ligand exchanges [17]. Polysaccharide-based CSPs are the most popular CSPs today. They demonstrated a successful chiral recognition ability, good chemical stability and high loadability in common separation modes such as polar organic, normal phase and reverse-phase chiral LC, particularly by optimising these separation conditions [18]. Zhang et al. [19] reported the ability of the polysaccharide-based CSP, 3,5-dimethylphenylcarbamate of amylose, in enantiomeric separations of more than 95% of racemic compounds in chromatographic techniques. However, it is not always predictable which type of CSP is able to enantiomerically separate a specific compound or class of compounds without being empirically tested under different conditions.

As a logical sequence based on previous investigations across many types of chiral columns using HPLC by our research group, CHIRALPAK® AD-3 comprising of amylose tris(3,5-dimethylphenylcarbamate) coated on 3-μm silica-gel was selected as stationary

3 phase. Three synthetic cathinones namely, 4-methylmethcathinone (also known as mephedrone), *N*-Ethylpentylone and 4-chloro-α-pyrrolidinopropiophenone (4-Cl-alpha-PPP) were used as a model compounds (Figure 1).

A mobile phase containing 0.1% diethylamine (DEA) as a basic modifier in methanol–ethanol with a starting ratio of (50:50, v/v) as recommended by the column manufacturer for alcohol mixtures. The effect of different mobile phase proportions on retention time and the resolution of the enantiomers is shown in Table 1.

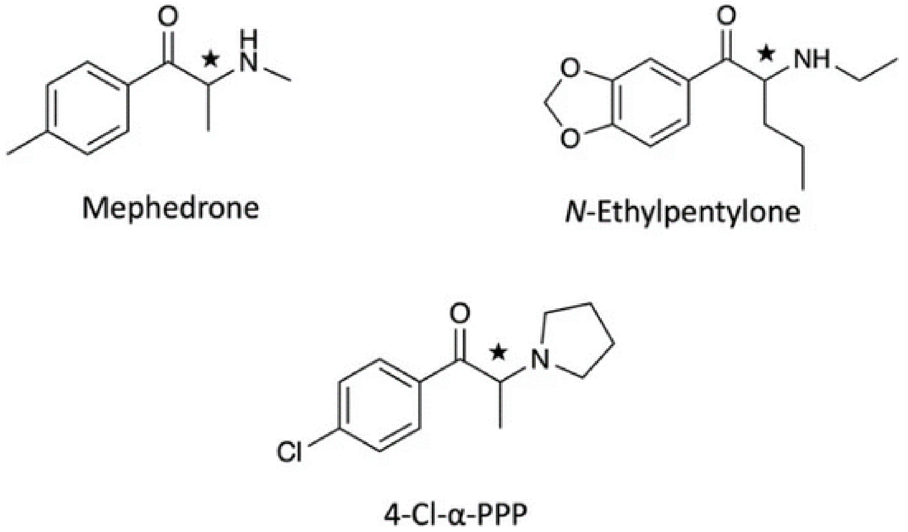

Figure 1: Synthetic cathinones used in this study. (★) denotes chiral center

	methanol–ethanol (80:20, v/v)			methanol–ethanol (95:5, v/v)		
	t_1	t_2	Resolution	t_1	t_2	Resolution
Mephedrone	5.24	6.17	1.10	5.31	6.58	1.58
N-Ethylpentylone	5.41	5.75	0.51	5.34	5.92	0.76
4-Cl-alpha-PPP	4.55	5.22	1.04	4.58	5.31	0.98

Where t_1 and t_2 are the retention times of first and second eluting peaks.

Table 1: Effect of different mobile phase ratios on the retention time and resolution of the synthetic cathinone enantiomers

As a result, a mobile phase with 80% content of methanol has shown a partial resolution of the three compounds as indicated in Figure 2. However, increasing the content of methanol turns out to be more effective. As shown in Figure 3, the partial resolution of mephedrone has transformed into a complete separation by increasing the methanol content from 80% to 95%. However, both 4-Cl-alpha-PPP and *N*-Ethylpentylone were only partially resolved although a remarkable resolution was observed.

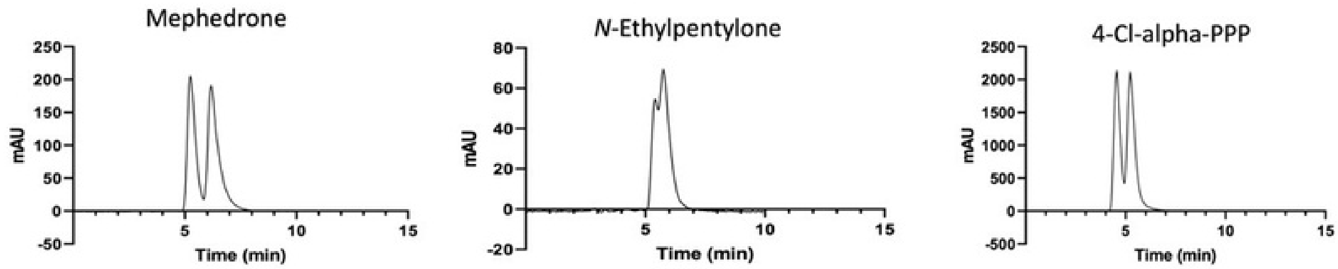

Figure 2: HPLC separation of the enantiomers of cathinone derivatives on a CHIRALPAK® AD-3 column with a flow rate of 0.1 ml/min and UV detection at 254 nm with methanol–ethanol–DEA (80:20:0.1 v/v)

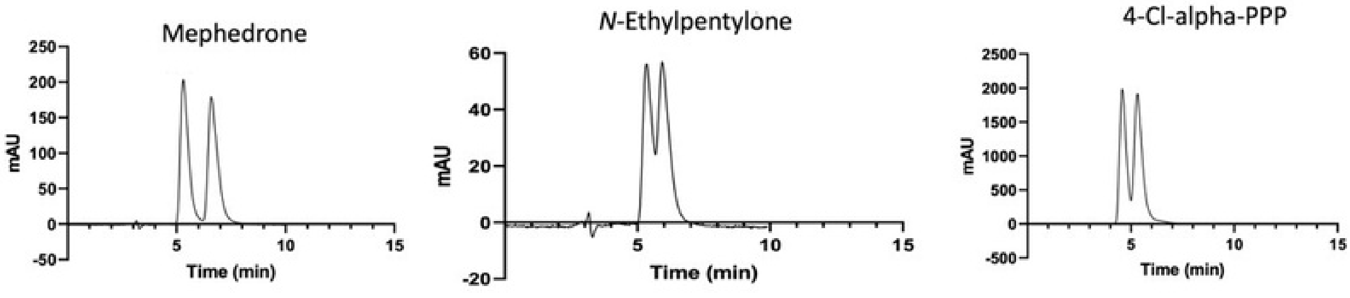

Figure 3: HPLC separation of the enantiomers of cathinone derivatives on a CHIRALPAK® AD-3 column with a flow rate of 0.1 ml/min and UV detection at 254 nm with methanol–ethanol–DEA (95:5:0.1 v/v)

It appears that under these conditions, the CHIRALPAK® AD-3 column is suitable for chiral resolution of the secondary amine derivative - mephedrone - but not with the methylenedioxy-substituted nor pyrrolidinyl included in a ring structure. Likely due to the inability of these groups to form hydrogen bondings with the chiral selector. This hypothesis can be reinforced in that the structure of mephedrone is rather similar to the structure of 4-Cl-alpha-PPP except for the presence of nitrogen atom.

In conclusion, with the continuous introduction of new cathinone derivatives to the illicit drug markets, it can be assumed that, despite their illegal status, these drugs may remain prevalent for the foreseeable future. The performance of the introduced chiral method was capable of enantioseparation of 1 out of 3 cathinone derivatives with an improved resolution for the rest of the compounds. Although the current method does not meet the aim to be suitable for baseline resolving through varied groups of synthetic cathinones, it leads to the hypothesis that the ability of secondary amines to form hydrogen bonding is mostly contributed to the appropriate interaction to the CSP. Nevertheless, the applicability of the developed chiral method could be expanded to a wider range of designer drugs to provide much more precise knowledge.

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References:

[1] Albals D, Heyden Y Vander, Schmid MG. Chiral Separations of Cathinone and Amphetamine-derivatives: Comparative Study Between Capillary Electrochromatography, Supercritical Fluid Chromatography and Three Liquid Chromatographic Modes. J Pharm Biomed Anal. 2016;121:232–243. doi:10.1016/j.jpba.2015.12.007

[2] Wsol V, Skalova L, Szotakova B. Chiral Inversion of Drugs: Coincidence or Principle? Curr Drug Metab. 2004;5:517–533. doi:10.2174/1389200043335360

[3] Erny GL, Cifuentes A. Liquid Separation Techniques Coupled with Mass Spectrometry for Chiral Analysis of Pharmaceuticals Compounds and Their Metabolites in Biological Fluids. J Pharm Biomed Anal. 2006;40:509–515. doi:10.1016/j.jpba.2005.10.044

[4] Tokunaga E, Yamamoto T, Ito E, Shibata N. Understanding the Thalidomide Chirality in Biological Processes by the Self-disproportionation of Enantiomers. Sci Rep. 2018;8:6–13. doi:10.1038/s41598-018-35457-6

[5] Bosch ME, Sánchez AJR, Rojas FS, et al. Recent Advances in Analytical Determination of Thalidomide and its Metabolites. J Pharm Biomed Anal. 2008;46:9–17. doi:10.1016/j.jpba.2007.10.003

[6] Dhabbah AM. Determination of Chiral Cathinone in Fresh Samples of Catha edulis. Forensic Sci Int. 2020;307:110105. doi:10.1016/j.forsciint.2019.110105

[7] Glennon RA, Martin BR, Dal Cason TA, et al. Methcathinone (“cat”): An Enantiomeric Potency Comparison. Pharmacol Biochem Behav. 1995;50:601–606. doi:10.1016/0091-3057(94)00348-3

[8] Kalix P. The Releasing Effect of the Isomers of the Alkaloid Cathinone at Central and Peripheral Catecholamine Storage Sites. Neuropharmacology. 1986;25:499–501. doi:10.1016/0028-3908(86)90174-7

[9] Islam MW, Tariq M, Ageel AM, et al. An Evaluation of the Male Reproductive Toxicity of Cathinone. Toxicology. 1990;60:223–234. doi:10.1016/0300-483X(90)90145-7

[10] Řezanka P, Macková D, Jurok R, et al. Enantioseparation and Determination of Mephedrone and Its Metabolites by Capillary Electrophoresis Using Cyclodextrins as Chiral Selectors. Molecules. 2020;25:2879. doi:10.3390/molecules25122879
[11] Peng L, Jayapalan S, Chankvetadze B, et al. Reversed-phase Chiral HPLC and LC/MS Analysis with Tris(chloromethylphenylcarbamate) Derivatives of Cellulose and Amylose as Chiral Stationary Phases. J Chromatogr A. 2010;1217:6942–6955. doi:10.1016/j.chroma.2010.08.075
[12] Mohr S, Taschwer M, Schmid MG. Chiral Separation of Cathinone Derivatives Used as Recreational Drugs by HPLC-UV Using a CHIRALPAK® AS-H Column as Stationary Phase. Chirality. 2012;24:486–492. doi:10.1002/chir.22048
[13] Weiß JA, Mohr S, Schmid MG. Indirect Chiral Separation of New Recreational Drugs by Gas Chromatography-Mass Spectrometry Using Trifluoroacetyl-L-Prolyl Chloride as Chiral Derivatization Reagent. Chirality. 2015;27:211–215. doi:10.1002/chir.22414
[14] Taschwer M, Seidl Y, Mohr S, et al. Chiral Separation of Cathinone and Amphetamine Derivatives by HPLC/UV Using Sulfated ß-Cyclodextrin as Chiral Mobile Phase Additive. Chirality. 2014;26:411–418. doi:10.1002/chir.22341
[15] Kadkhodaei K, Kadisch M, Schmid MG. Successful Use of a Movel lux® i-Amylose-1 chiral Column for Enantioseparation of "legal highs" by HPLC. Chirality. 2020;32:42–52. doi:10.1002/chir.23135
[16] Almeida AS, Silva B, Pinho PG de, et al. Synthetic Cathinones: Recent Developments, Enantioselectivity Studies and Enantioseparation Methods. Molecules. 2022;27:2057. doi:10.3390/molecules27072057
[17] Silva B, Fernandes C, Guedes de Pinho P, et al. Chiral Resolution and Enantioselectivity of Synthetic Cathinones: A Brief Review. J Anal Toxicol. 2018;42:17–24. doi:10.1093/jat/bkx074
[18] Schwaninger AE, Meyer MR, Maurer HH. Chiral Drug Analysis Using Mass Spectrometric Detection Relevant to Research and Practice in Clinical and Forensic Toxicology. J Chromatogr A. 2012;1269:122–135. doi:10.1016/j.chroma.2012.07.045
[19] Zhang T, Kientzy C, Franco P, et al. Solvent Versatility of Immobilized 3,5-Dimethylphenylcarbamate of Amylose in Enantiomeric Separations by HPLC. J Chromatogr A. 2005;1075:65–75. doi:10.1016/j.chroma.2005.03.116

Cannabis-Based Therapy for Depression

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Depression is one of the most common and serious mental disorders that impacts almost 280 million people globally [1]. Individuals suffering from depression may exhibit symptoms such as persistent emotions of sadness or low mood, an absence of interest or enjoyment, difficulty sleeping and decreased focus. If these symptoms continue or occur continually, they typically result in significant limitations in an individual's ability to perform their daily life activities. Depression, at its worst, can result in suicide [2,12,13]. The cause of major depressive illness is complex, involving a combination of genetic and environmental variables. Individuals who have first-degree relatives with depression have a threefold increased likelihood of developing depression compared to the general population. However, it is important to note that depression can also emerge in persons without a family history of the condition [11]. Depression is commonly treated with antidepressant drugs and psychological treatments such as group or individual therapy sessions. Both techniques have shown effectiveness, however their impact may not significantly vary from placebo in mild-to-moderate cases. Empirical data indicates that the combined use of these therapies may yield the most favourable results over time.

However, a significant proportion of individuals fail to respond to either method, resulting in chronic symptoms. Patients exhibit diverse prescription behaviours and recovery rates, suggesting that the efficacy of antidepressants is greatly impacted by patient factors such as alcohol or substance abuse, cardiovascular disease, metabolic disorders, age and gender [3,15,16]. Predicting the most effective treatment for an individual continues to be a difficult task and there have been no notable advancements in treatment alternatives over the past two decades [3][14]. These significant gaps in depression treatment have led to interest in alternative therapies, including the study of cannabinoids like THC and CBD [17,4]. Among these, the endocannabinoid system has emerged as a significant area of research, highlighting the potential use of cannabinoids in the treatment of depression [4].

Cannabis, sometimes referred to as marijuana is a group of flowering plants with a long history of therapeutic use over thousands of years [18]. The group includes various species, most notably *Cannabis sativa* and *Cannabis indica*, which are extensively utilized for both medicinal and recreational purposes [5,19]. *Cannabis sativa* strains are known for their energetic impact on individuals, frequently boosting creativity and concentration, therefore making them suitable for daytime use [20]. On the other hand, *Cannabis indica* strains typically have a more calming effect, making them appropriate for nighttime use to help with relaxation and sleep [21]. Cannabis is composed of more than 400 substances, including flavonoids, terpenoids, and cannabinoids. Terpenes found in cannabis are aromatic compounds that enhance the unique smell and flavour of the plant. Additionally, they have a vital function in therapeutic outcomes, amplifying the advantages of cannabinoids via the "entourage effect," which may assist in regulating mood, reducing anxiety and promoting anti-inflammatory reactions [31].

The most notable components found in cannabis are THC (Δ-9 tetrahydrocannabinol) and CBD (cannabidiol) [5]. THC is the main chemical compound of the plant that is responsible for its psychoactive effects. It can cause a range of mood changes from feelings of elation to anxiety or paranoia. Cannabinoids undergo hepatic metabolism by cytochrome P450 enzymes [18]. The ECS includes cannabinoid receptors CB1 and CB2, endocannabinoids such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG) and enzymes responsible for the production and breakdown of endocannabinoids.

The CB1 receptor is primarily associated with the Central Nervous System (CNS), whereas the CB2 receptor is mainly associated with the immune system [17]. Typically, the speed, potency and type of cannabis effects experienced by users vary depending on the method of administration. Smoking cannabis, whether it be marijuana or another type of cannabis, is the most direct way of THC administration. However, pure, and FDA-approved therapeutic formulations of THC can be obtained in the form of pills [23]. THC primarily acts by mimicking endocannabinoids and acting as an agonist at both CB1 and CB2 receptors [22]. After ingestion, THC binds directly to CB1 receptors located in the brain. These receptors play a key role in controlling the release of neurotransmitters like GABA or glutamate [23,24]. THC alters the release patterns of several neurotransmitters by activating these receptors and affecting their normal regulatory functions. The alterations in neurotransmitter activities are responsible for the particular psychoactive effects of cannabis, including changed perception, changes in mood and decreased memory [7].

Although there has been plenty of research on the importance of the endocannabinoid system in depression [7,17], only a limited number of studies have specifically investigated the impact of external cannabinoids on depression [6]. The absence of research in this area is further complicated by different perspectives on cannabis, in part because of its potential for abuse. The effects of administering Cannabis mixtures or pure THC have been studied in laboratory animals, primarily in rats and mice, which have shown consistent patterns of behavior [8]. A study has investigated the antidepressant effects of Δ9-THC and other cannabinoids obtained from *Cannabis sativa* [6].

The study used male mice that were eight weeks old and included the Forced Swim Test (FST) and the Tail Suspension Test (TST), two well-established experimental techniques used to evaluate the efficacy of antidepressant medication in rodents, to assess antidepressant properties. The FST involves placing animals into a water tank and measuring their ability to escape. The TST involves hanging mice by their tails to assess their behaviors related to escape [17]. On the test day, the pre-injection control values for rectal temperature, catalepsy and tail flick latencies were measured. The animals received intraperitoneal injections (i.p.) of either the vehicle control (a mixture of ethanol, Cremophor and saline at a ratio of 1:1:18), the test drug (at doses ranging from 1.25 to 80 mg/Kg) or a control antidepressant (desipramine or fluoxetine, at doses ranging from 10 to 40 mg/Kg). A dose of 2.5 mg/Kg of Δ9-THC was administered to prevent the development of hypothermic or cataleptic effects. Both tests showed results indicating a decrease in immobility time, suggesting potential antidepressant effects [6]. Results such as those demonstrated in the study discussed above improve our understanding and appreciation of THC's possible antidepressant properties. Anecdotal reports indicate that certain individuals use cannabis as an effective therapy for depressive and manic symptoms [25][26][28]. For instance, Gruber et al. [27] documented five cases in which patients indicated that cannabis relieved their depression symptoms and that they intentionally used it for this specific reason. Furthermore, a cross-sectional study showed that those who engage in occasional or daily cannabis use have reduced levels of depressive symptoms compared to those who have never experimented with cannabis [28].

Recent research has also revealed a connection between chronic inflammation and depression [29]. Chronic inflammation can potentially induce depression by influencing the brain functioning and the immune system response [9]. Chronic inflammation produces inflammatory cytokines, including TNF-alpha, IL-1 and IL-6, which affect neurotransmitter systems and increase the body's stress response. Studies where healthy subjects are administered endotoxin infusions to stimulate the release of cytokines showed classical depressive symptoms emerge [30].

These alterations could inhibit typical brain functioning and affect behaviours, hence leading to the development of depression [9]. It has been suggested that THC possesses anti-inflammatory properties which are achieved by regulating the expression of important pro-inflammatory cytokines [10]. Cannabis extracts containing high THC concentrations have been found to effectively reduce the expression of cytokines such as COX2 and IL-6 in specific studies [10]. This effect becomes stronger when these extracts are combined with specific terpenes, which can amplify the anti-inflammatory properties of THC. In earlier research involving human peripheral blood mononuclear cells, THC and CBD at concentrations equivalent to plasma levels before smoking marijuana (10–100 ng/mL) were found to enhance the concentration of IFNγ. However, at higher concentrations (5–20 µg/mL), they completely inhibited the production and release of this cytokine [32]. Additionally, another study highlighted the biphasic impact of THC on the modulation of cytokines in mononuclear cells. The synthesis of TNF-α and IL-6 was suppressed by a concentration of 3 nM THC, but enhanced by a concentration of 3 µM THC, similar to the effect observed on IFNγ synthesis highlighting the complexity of its effects, which can vary depending on the concentration [33]. THC can relieve depressive symptoms linked with chronic inflammation by regulating inflammatory markers such as TNF-alpha, IL-1, and IL-6 [10].

In conclusion, it is suspected that THC has the potential to be used as a therapeutic agent against depression because it can affect the endocannabinoid system and create effects similar to antidepressant drugs in animals [6]. Currently, THC is not an approved alternative treatment for depression, and it is expected that its legal status as an illegal substance in many countries will delay further research and clinical studies to evaluate its true potential against depression as well as its therapeutic effectiveness, safety profile and recommended dose. The findings presented in this paper support the concept that cannabis may have a direct antidepressant effect. If verified, this phenomenon holds significant therapeutic significance as it might potentially lead to a substantial increase in the number of depressed individuals resorting to self-medication with cannabis [25].

Acronyms:

THC - Δ-9 tetrahydrocannabinol
CBD - Cannabidiol
ECS - Endocannabinoid System
AEA - Anandamide
2-AG - 2-arachidonoylglycerol
CNS - Central Nervous System
FDA - Food and Drug Administration
FST - Forced Swim Test
TST - Tail Suspension Test
COX2 - Cyclooxygenase-2
IL-6 - Interleukin-6
IFNγ - Interferon-gamma
TNF-α - Tumor Necrosis Factor-alpha
IL-1 - Interleukin-1
nM - Nanomolar (a concentration unit)
µM - Micromolar (a concentration unit)
ng/mL - Nanograms per milliliter (a concentration unit)
µg/mL - Micrograms per milliliter (a concentration unit)
mg/kg - Milligrams per kilogram (a dosage unit)
CB1 - Cannabinoid receptor type 1
CB2 - Cannabinoid receptor type 2
GABA - Gamma-Aminobutyric Acid

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References:

[1]. World Health Organization. Mental disorders [Internet]. Geneva: World Health Organization; 2022 [cited 2024 Apr 20]. Available from: https://www.who.int/news-room/fact-sheets/detail/mental-disorders
[2]. Bhowmik D, Sampath Kumar KP, Srivastava S, et al. Depression – Symptoms, Causes, Medications, and Therapies. Pharma Innovation. 2012;1:37-45
[3]. Alemi F, Min H, Yousefi M, et al. Effectiveness of common antidepressants: a post market release study. EClinicalMed. 2021;41:101171
[4]. García-Gutiérrez MS, Navarrete F, Gasparyan A, et al. Cannabidiol: A potential new alternative for the treatment of anxiety, depression, and psychotic disorders. Biomolecules. 2020;10:1575
[5]. Ebbert JO, Scharf EL, Hurt RT. Medical cannabis. Mayo Clin Proc. 2018;93(12):1842-1847
[6]. El-Alfy AT, Ivey K, Robinson K, et al. Antidepressant-like effect of Δ9-tetrahydrocannabinol and other cannabinoids isolated from Cannabis sativa L. Pharmacol Biochem Behav. 2010;95(4):434-442
[7]. Huang WJ, Chen WW, Zhang X. Endocannabinoid system: Role in depression, reward and pain control (Review). Mol Med Rep. 2016;14(4):2899-903
[8]. Alexander SPH. Therapeutic potential of cannabis-related drugs. Prog Neuro-Psychopharmacol Biol Psychiatry. 2016;64:157-166
[9]. Leonard BE. Inflammation and depression: a causal or coincidental link to the pathophysiology? Acta Neuropsychiatr. 2018;30(1):1-16
[10]. Li D, Ilnytskyy Y, Ghasemi Gojani E, et al. Analysis of Anti-Cancer and Anti-Inflammatory Properties of 25 High-THC Cannabis Extracts. Molecules. 2022;27(18):6057
[11]. Chand SP, Arif H, Kutlenios RM. Depression (Nursing). In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024. Chapter Depression (Nursing); p. Available from: https://www.ncbi.nlm.nih.gov/books/NBK568733/
[12]. National Institute of Mental Health. Depression [Internet]. Bethesda (MD): National Institute of Mental Health; [cited 2024 Apr 20]; [about 2 screens]. Available from: https://www.nimh.nih.gov/health/topics/depression

[13]. Penninx BW, Milaneschi Y, Lamers F, et al. Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile. BMC Med. 2013;11:129
[14]. Roiser J, Elliott R, Sahakian B. Cognitive mechanisms of treatment in depression. Neuropsychopharmacol. 2012;37:117-136
[15]. Sheffler ZM, Patel P, Abdijadid S. Antidepressants. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538182/
[16]. Hengartner MP. How effective are antidepressants for depression over the long term? A critical review of relapse prevention trials and the issue of withdrawal confounding. Ther Adv Psychopharmacol. 2020;10:2045125320921694
[17]. Hasbi A, Madras BK, George SR. Endocannabinoid system and exogenous cannabinoids in depression and anxiety: a review. Brain Sci. 2023;13:325
[18]. Turner AR, Patel P, Agrawal S. Marijuana. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430801/
[19]. Atakan Z. Cannabis, a complex plant: different compounds and different effects on individuals. Ther Adv Psychopharmacol. 2012;2(6):241-254
[20]. Saleh N. Indica vs. Sativa: Understanding the Differences [Internet]. Verywell Health; 2024 May 28 [cited 2024 Jun 26]; [about 2 screens]. Available from: https://www.verywellhealth.com/indica-vs-sativa-1123887
[21]. Schilling S, Melzer R, McCabe PF. Cannabis sativa. Curr Biol. 2020;30
[22]. Capodice JL, Kaplan SA. The endocannabinoid system, cannabis, and cannabidiol: implications in urology and men's health. Curr Urol. 2021;15(2):95-100
[23]. Alger BE. Getting high on the endocannabinoid system. Cerebrum. 2013;2013:14
[24]. Hoffman AF, Lupica CR. Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. J Neurosci. 2000;20(7):2470-2479
[25]. Gruber AJ, Pope HG Jr, Brown ME. Do patients use marijuana as an antidepressant? Depression. 1996;4(2):77-80
[26]. Ashton CH, Moore PB, Gallagher P, Young AH. Cannabinoids in bipolar affective disorder: a review and discussion of their therapeutic potential. J Psychopharmacol. 2005;19(3):293-300
[27]. Denson TF, Earleywine M. Decreased depression in marijuana users. Addict Behav. 2006;31(4):738-742
[28]. Scherma M, Masia P, Deidda M, Fratta W, Tanda G, Fadda P. New perspectives on the use of cannabis in the treatment of psychiatric disorders. Medicines. 2018;5:107
[29]. Berk M, Williams LJ, Jacka FN, et al. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med. 2013;11:200
[30]. Reichenberg A, Yirmiya R, Schuld A, et al. Cytokine-associated emotional and cognitive disturbances in humans. Arch Gen Psychiatry. 2001;58(5):445-452
[31]. Hanuš LO, Hod Y. Terpenes/terpenoids in cannabis: are they important? Med Cannabis Cannabinoids. 2020;3(1):25-60
[32]. Zádor F, Joca S, Nagy-Grócz G, et al. Pro-inflammatory cytokines: potential links between the endocannabinoid system and the kynurenine pathway in depression. Int J Mol Sci. 2021;22:5903
[33]. Berdyshev EV, Boichot E, Germain N, et al. Influence of fatty acid ethanolamides and Δ9-tetrahydrocannabinol on cytokine and arachidonate release by mononuclear cells. Eur J Pharmacol. 1997;330(2):231-240

Science in Motion

This next section publishes peer reviewed articles for our readership from other disciplines

Applications of Predictive Modelling in Biomedical Engineering: A Case Study of the Porosity of Hydroxyapatite Pellets

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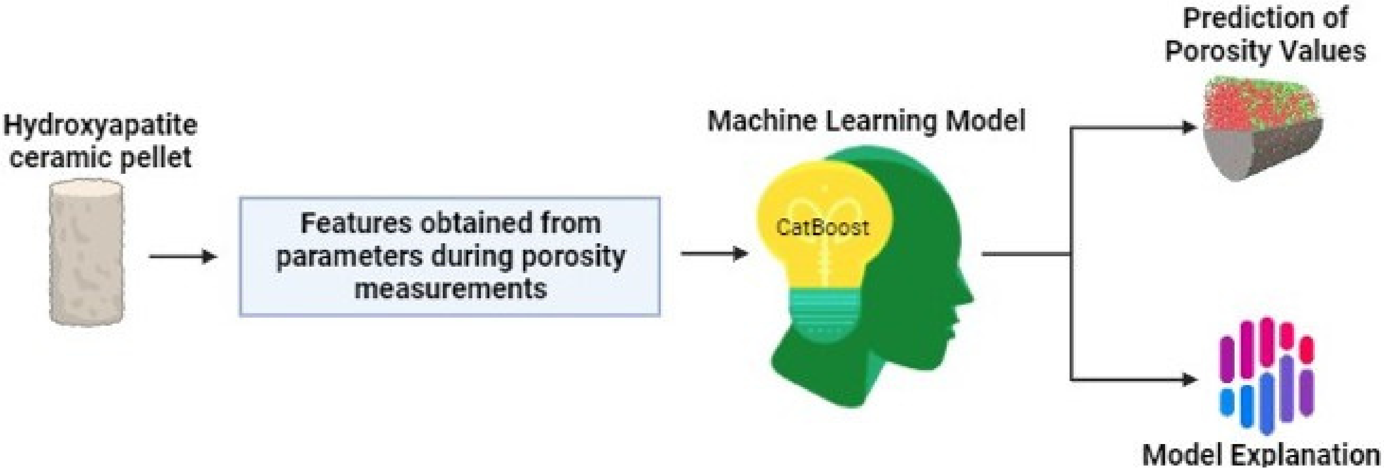
Keywords: Hydroxyapatite, Machine Learning, Datasets, Porosity, Modelling



Introduction:

In recent years, the significance of employing porous hydroxyapatite (HA) in bone replacement surgeries has been on the rise [1,2]. The size, shape and pattern of pores are important factors for osteoconduction and the inclusion of pores is crucial [3]. In bone regeneration, HA is commonly designed to possess a notable combination of micro and macro porosities which facilitates the formation of tissues and their proliferation. Highly porous scaffolds are essential for cell infiltration, attachment, high surface area-to-volume ratio and minimal diffusion constraints during cell culture. Hence, to guarantee a consistent microstructure and desirable porosity, the isostatic pressing technique has been reported as a viable strategy to attain uniformity in compacting HA [4]. This technique, a powder metallurgy process, ensures that pressure is evenly distributed across a powder compact, allowing for optimal density and microstructure attainment without constraints posed by geometry.

Previous studies have demonstrated that the utilization of porous HA for bone repair, possessing appropriate pore quantities and dimensions which can facilitate the proliferation of osteoblasts and blood vessels, hence expediting the process of bone regeneration. In addition, porous HA ceramics provide favourable osteogenic induction capabilities and exhibit an enhanced ability to resolve bone defects, hence aligning more closely with the therapeutic demands for biological bone repair materials [5]. Furthermore, when considering these practical applications, it is important to note that mechanical properties like compressive strength are inversely proportional to the volume of macro-porosity. A demerit of porous HA scaffolds is the reduction in the mechanical properties. Therefore, it is important to optimize the porosity and mechanical properties of the scaffold especially for load bearing bone repair applications. Developing porous implant materials that closely resemble the bimodal structure of bone with a satisfactory level of interconnectivity is a problem.

The software possesses two primary attributes: the capacity to handle categorical data, denoted as "the Cat," and the utilisation of gradient boosting, referred to as "the Boost." The process of gradient boosting entails the repetitive development of several decision trees (multiple base learners). What sets it apart from other boosting algorithms is its use of symmetric trees and oblivious decision trees. In each step, the algorithm selects the feature-split pair with minimal loss as determined by a penalty function and applies it to all nodes in the level. This results in a balanced tree that allows for efficient CPU implementation, faster prediction times and reduced overfitting. The accuracy of subsequent trees is enhanced by using the outcomes of preceding trees. The CatBoost algorithm improves upon the original gradient-boosting approach to expedite its implementation [12].

CatBoost is a distinctive approach based on decision trees that streamlines the process of data pre-processing. The model has the capability to efficiently process a mixture of categorical and non-category variables by employing ordered encoding to substitute categorical characteristics. One distinguishing characteristic of CatBoost is the utilisation of symmetric trees, whereby decision nodes at each level of depth employ identical split conditions. This ML method compares favorably to XGBoost and LightGBM in computational efficiency and performance [16,17].

It efficiently handles categorical features without extensive pre-processing and shows competitive training speed. While efficiency may vary, CatBoost demonstrates robust performance across diverse datasets, including those with heterogeneous features and noise. Researchers successfully leverage CatBoost for tasks involving imbalanced data, highlighting its effectiveness in addressing class distribution issues [18].

Moreso, CatBoost stands out for its efficient feature handling, competitive training speed, strong performance across datasets and effectiveness in managing imbalanced data, offering a versatile solution for various ML applications, while simultaneously preserving crucial attributes like cross-validation, regularisation, and support for missing values from previous algorithms. To prevent overfitting, CatBoost incorporates the ordered boosting technique, which trains the model on a subset of the data while computing residuals on a different subset. Furthermore, CatBoost demonstrates strong performance across datasets of varying sizes, including both small and large datasets [13]. Overall, CatBoost is a powerful and efficient ML tool capable of handling even the most complex datasets with ease. This has been used in several bioengineering applications [19-21].

Preliminary Results and Discussion:

The calculated porosity can be influenced by 7 factors viz: volume of the pores, weight of water retained in the pores of the pellets, bulk volume of each pellet, weight of the dry and saturated pellets (dry and wet mass), height, diameter and radius of the pellets [2,4]. These factors were selected as features/independent variables (Figure 1c) and passed to the CatBoost ML algorithm for the prediction tasks with random effects (the random effect with the best coefficient of determination score R^2 is presented). There were 17 examples and 7 input features. The data was split into a training set (80%) and a testing set (20%). The test set is usually employed for the evaluation of the model's accuracy.

To assess the ML method, the coefficient of determination R^2 was employed as given by equation 2:

$$R^2 = 1 - \frac{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{N}}{\frac{\sum_{i=1}^{N}(y_i - \bar{y})^2}{N}}. \qquad (2)$$

It is assumed that the dataset contains N samples, is the predicted value for the i-th data point, is the known value for the i-th data point, t,yi is the known value for the i-th data point. y with the hat (predicted) and no hat (known value).

Presently, porosity measurement that utilises the Archimedes principle is common, but its accuracy can be influenced due to various experimental protocols [6]. The absolute errors in these measurements may be minimized by using machine learning (ML) models.

The substantial surge of data in science and engineering, emanating from both experimental work and theoretical frameworks, has facilitated the increasingly prevalent ability of researchers to predict interesting properties of innovative and intelligent materials. ML, an artificial intelligence subfield, has undergone substantial growth in recent years. It is largely interdisciplinary being a combination of mathematics, statistics, computer science, programming and domain-specific knowledge. Owing to its robust data processing capabilities, ML serves as an effective means to reduce human and material costs in the process of novel material development, and reduces the research and development cycle. ML can be categorized into four main learning tasks: supervised learning, unsupervised learning, semi-supervised learning and reinforcement learning.

A typical supervised learning (SL) involves developing an ML model from labelled data which has found use in solving classification and regression tasks. Some examples of SL models include Linear Regression, Logistic Regression, Decision Trees, Random Forest, Neural Networks, Light Gradient Boosting Machine (LightGBM), Categorical Boosting (CatBoost) to mention just a few.

Machine learning has found applications in various fields, including biology and chemistry [7,8]. Many ML algorithms provide a scientific basis for establishing intrinsic insights about a data and the underlying correlation between the experimental parameters and the physical characteristics of materials. By including experimental parameters in the generated model, it becomes feasible to predict physical properties before conducting actual trials. The utilization of ML has demonstrated the effectiveness of handling a wide range of experimental scenarios, providing the benefit of rapid predictions. Consequently, this can result in decreased development timelines and lowered expenses associated with experimentation.

Despite the potential benefits of ML in bioengineering, its application has been limited due to the lack of available datasets in this field of study. Small datasets are prevalent in biomedical engineering due to the complexity and high cost of experiments, which limits the amount of available data. The limited data size (small dataset) can result in the creation of models with limited generalization prowess when evaluated on new test examples.

Cross-validation may become unreliable when the test size is small. This is a caveat and some studies are proposing new ideas for validating ML models with random effects due to small data. Shaikhina et al. [9] conducted a study using a neural network and a decision tree with a sample size of 35 in the field of tissue engineering which involved both regression and classification tasks. Despite the small datasets used, the models yielded an efficiency of 98.3% and 85%, respectively.

While previous studies have separately investigated the application of ML models in predicting structural features of substituted HA and bone formation rates [10,11], this report presents an examination of porosity measurement in HA ceramics coupled with the utilization of the CatBoost ML model for predicting porosity values. This case report provides insights into the possibility of predicting, for the first time, the porosity of HA pellets with random effects despite the small datasets. It is understood that impracticability may exist, however, with the results presented, it is possible to propose a pathway for a generalized framework for using small datasets for predictive modelling in bioengineering.

Data and Model:

Porosity is a quantifiable characteristic that denotes the extent of empty spaces within a certain material, expressed as a ratio of the volume occupied by these voids to the overall volume of the material. By careful design of experiments, response surface methodology (RSM) was used to optimize the properties (porosity inclusive) of the HA material.

The aim was to optimize a response variable affected by multiple independent variables. The RSM optimization technique, as designed, produced 17 iterations/HA compositions.

For this study, the porosity values of the 17 HA compositions were determined experimentally using equation 1[15], which is based on Archimedes' principle, and the datasets (17) was used for the ML predictions.

$$\text{Porosity} = \left(\frac{W_2 - W_1}{W_2 - W_3}\right),$$

where: W_1 = Weight of the sample in air, W_2 = weight of the wet sample after soaking for 24 h, and W_3 = weight of the sample suspended in water.

CatBoost ML regression model:

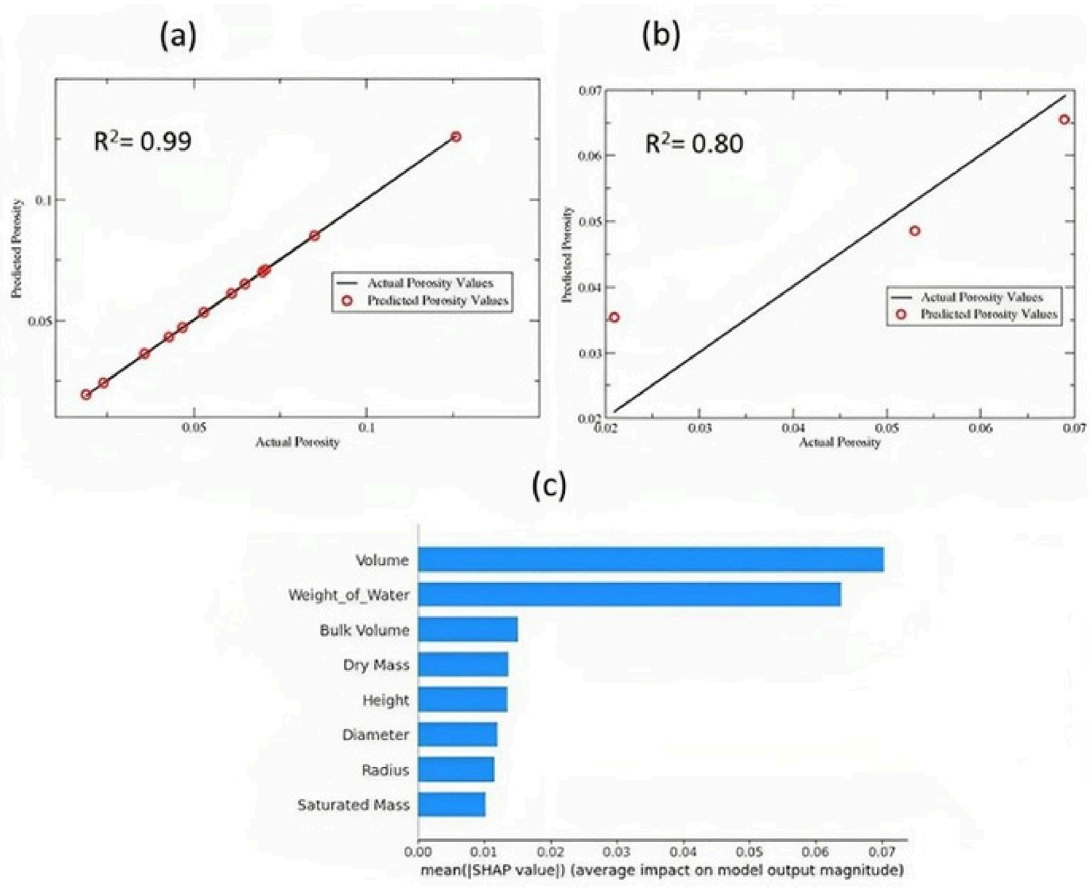
The CatBoost technique is a popular method that uses base learners to handle categorical features and make accurate regression predictions. It employs a unique learning procedure that involves organizing and applying these features. This ensemble learning approach has several advantages, including an efficient training process, regularization techniques, and the ability to handle missing data, all of which contribute to its robust performance. Additionally, built-in cross-validation makes hyperparameter tuning easier, while its scalability allows for training on both large and small datasets. This particular ML method represents one of the most competitive and advanced ensemble learning techniques currently available. It uses a combination of multiple base learners for the purpose of performing classification and regression prediction of a target label from a given data distribution.

The SHAP (Shapley Additive exPlanations) framework was adopted to understand the importance of the input features. The framework was developed in 2017 [14] and it helps understand the significance of input features passed to a ML model. SHAP is based on fair profit allocation among numerous stakeholders based on their contribution. This framework explains the predictions of ML models in a way that is easy to understand.

The explainability scheme (SHAP) aid in interpreting the rationale influencing the supervised learning decision: this is done by providing information about the ranking of the input feature importance and overall impact to the prediction of the target output label (porosity of HA). Figure 1(a) and 1(b), show that the CatBoost model yielded a coefficient of determination (R^2 = 0.99) in the training phase which indicate a strong predictive capability. To gain more insight about the model goodness and generalization capability, we evaluated the model on the testing examples which yielded (R^2 = 0.80) and this depicts an acceptable level of predictive strength.

It should however be noted that the results presented here only points to the possibility that the ML models can minimize the experimental complexities, time and expense during porosity measurements of biomaterials. The approach presented can be improved if the RSM models used can generate more data points such that cross-validation can be employed during the model validation. The SHAP analysis (Figure 1c) revealed that the volume of the pores, weight of water retained in the pores and bulk volume of the pellets were the most important features that determined the prediction of the porosity values. Given that porosity is typically the percentage of voids in a sample (i.e., the ratio of the volume of the voids divided by the total volume), this feature (volume of the pores) can be considered as extremely important and is confirmed by the SHAP analysis. These results not only highlight the most important features but also showcase that RSM methods along with ML models can be further explored in the predictions of porosity values without performing the time-consuming experiments.

Figure 1: Correlation plot for training set. (b) Correlation plot for testing sets (c) SHAP plot.



Conclusions:

Experimental data obtained from experimental porosity measurements were utilised to establish a CatBoost regression model. The results of the training set show that the model matches well with experimental data and this can be used as a reference for materials design. The impact of ML performance resulting from a small number of samples is highlighted and this can be alleviated by using large datasets or using surrogate data for the validation of the regression model. Some studies have proposed a methodology of doing many iterations which can be facilitated by the establishment of reliable comparisons among different ML configurations.

Acronmyns:

Hydroxyapatite (HA)
Hydroxyapatite, also known as hydroxylapatite, is a naturally occurring form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$ often written $Ca_{10}(PO_4)_6(OH)_2$ indicating two entities in the crystal unit cell.

Machine Learning (ML)
Machine learning refers to the use and advancement of computer systems that possess the capability to acquire knowledge and adjust their behaviour without relying on explicit instructions. This is achieved through the implementation of algorithms and statistical models, which enable the analysis and deduction of patterns from data.

Supervised Learning (SL)
Supervised learning is a subset of machine learning that used labelled datasets to train algorithms to make predictions and identify patterns. Supervised learning algorithms are provided with labelled training data to acquire knowledge about the relationship between the input and output variables.

Response Surface Methodology (RSM)
Response Surface Methodology (RSM) is a set of mathematical and statistical approaches used to model and analyse intricate interactions between numerous independent factors and the resulting reactions on the dependent variable.

Categorical Boosting (CatBoost)
CatBoost is a supervised machine learning technique. It utilises decision trees for both classification and regression tasks. CatBoost is a machine learning algorithm that specialises in handling categorical data and utilises gradient boosting techniques.

SHAP (Shapley Additive exPlanations)
SHAP (SHapley Additive exPlanations) values provide a method for elucidating the results of machine learning models. The approach employed utilises game theory to quantify the individual contribution of each participant to the ultimate result.

References:

[1] Liu DM. Influence of porosity and pore size on the compressive strength of porous hydroxyapatite ceramic. Ceramics International. 1997;23(2):135-9.
[2] Obada DO, Dauda ET, Abifarin JK, Dodoo-Arhin D, Bansod ND. Mechanical properties of natural hydroxyapatite using low cold compaction pressure: effect of sintering temperature. Materials Chemistry and Physics. 2020;239:122099.
[3] Babaie E, Bhaduri SB. Fabrication aspects of porous biomaterials in orthopedic applications: a review. ACS Biomaterials Science & Engineering. 2018;4(1):1-39.
[4] Akpan ES, Dauda M, Kuburi LS, Obada DO. Bio-inspired synthesis of hydroxyapatite materials from two natural sources: a crack behavior and biological insight. Journal of the Australian Ceramic Society. 2023;1-9.
[5] Bie H, Chen H, Shan L, Tan CY, Al-Furjan MSH, Ramesh S, et al. 3D Printing and Performance Study of Porous Artificial Bone Based on HA-ZrO2-PVA Composites. Materials. 2023;16(3):1107.
[6] Akpan ES, Dauda M, Kuburi LS, Obada DO, Dodoo-Arhin D. A comparative study of the mechanical integrity of natural hydroxyapatite scaffolds prepared from two biogenic sources using a low compaction pressure method. Results in Physics. 2020;17:103051.
[7] Beč A, Mioč M, Bertoša B, Kos M, Debogović P, Kralj M, et al. Design, synthesis, biological evaluation and QSAR analysis of novel N-substituted benzimidazole derived carboxamides. Journal of Enzyme Inhibition and Medicinal Chemistry. 2022;37(1):1327-39.
[8] Yada A, Nagata K, Ando Y, Matsumura T, Ichinoseki S, Sato K. Machine learning approach for prediction of reaction yield with simulated catalyst parameters. Chemistry Letters. 2018;47(3):284-7.
[9] Shaikhina T, Lowe D, Daga S, Briggs D, Higgins R, Khovanova N. Machine Learning for Predictive Modelling based on Small Data in Biomedical Engineering. 2015.
[10] Yu J, Wang Y, Dai Z, Yang F, Fallahpour A, Nasiri-Tabrizi B. Structural features modeling of substituted hydroxyapatite nanopowders as bone fillers via machine learning. Ceramics International. 2021;47(7):9034-47.
[11] Wu C, Entezari A, Zheng K, Fang J, Zreiqat H, Steven GP, et al. A machine learning-based multiscale model to predict bone formation in scaffolds. Nature Computational Science. 2021;1(8):532-41.
[12] Dorogush AV, Ershov V, Gulin A. CatBoost: gradient boosting with categorical features support. arXiv preprint arXiv:1810.11363. 2018.
[13] Prokhorenkova L, Gusev G, Vorobev A, Dorogush AV, Gulin A. CatBoost: unbiased boosting with categorical features. Advances in neural information processing systems. 2018;31.
[14] Lundberg SM, Lee SI. A unified approach to interpreting model predictions. Advances in neural information processing systems. 2017;30.
[15] Zreiqat H, Ramaswamy Y, Wu C, Paschalidis A, Lu Z, James B, et al. The incorporation of strontium and zinc into a calcium–silicon ceramic for bone tissue engineering. Biomaterials. 2010;31(12):3175-84.
[16] Prokhorenkova L, Gusev G, Vorobev A, Dorogush AV, Gulin A. CatBoost: unbiased boosting with categorical features. In: Bengio S, Wallach H, Larochelle H, Grauman K, Cesa-Bianchi N, Garnett R, editors. Advances in Neural Information Processing Systems 31. Curran Associates, Inc; 2018. p. 6638-48.
[17] Anghel A, Papandreou N, Parnell T, Palma A, Pozidis H. Benchmarking and optimization of gradient boosting decision tree algorithms. 2018.
[18] Hancock JT, Khoshgoftaar TM. CatBoost for big data: an interdisciplinary review. Journal of big data. 2020;7(1):1-45.
[19] Rafieyan S, Vasheghani-Farahani E, Baheiraei N, Keshavarz H. MLATE: Machine learning for predicting cell behavior on cardiac tissue engineering scaffolds. Computers in Biology and Medicine. 2023;158:106804.
[20] Ke L, Elibol A, Wei X, Cenyu L, Wei W, Chong NY. Machine Learning Algorithm to Predict Cardiac Output Using Arterial Pressure Waveform Analysis. In: 2022 IEEE International Conference on Bioinformatics and Biomedicine (BIBM). 2022. p. 1586-91.
[21] Al-Hussaini I, Mitchell CS. SeizFt: Interpretable Machine Learning for Seizure Detection Using Wearables. Bioengineering. 2023;10(8):918.

Using your smart devices in the kitchen: A Recipe for Contamination?

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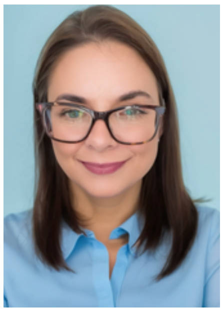

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Keywords: Smart Devices, Cooking, Food Safety, Microbial Contamination, Pathogenic Bacteria, Tablets, Mobile Phones, Cross-Contamination, Foodborne Disease

In today's digital age, where technology seamlessly integrates into our daily lives, even the most traditional spaces like the kitchen are not spared. From searching for recipes to watching cooking tutorials, capturing moments for social media, smart devices have become indispensable tools for many during meal preparation. A recently published research project commissioned by safefood investigated the use of smart devices and food preparation in domestic kitchens across the island of Ireland. Led by a team from Queen's University Belfast in Northern Ireland, this study revealed important insights into consumer behaviour and associated microbiological food safety risks.

Understanding Consumer Behaviour:

Observations from the in-kitchen study revealed a significant reliance on smart devices during meal preparation, with smartphones emerging as the device of choice for most participants. However, what caught the researchers' attention was the fluctuating adherence to food safety practices. Despite the awareness of potential hazards, such as cross-contamination, observed behaviours often fell short of safe practices. Notably, hand hygiene, particularly after handling raw ingredients like chicken and eggs, was found lacking in a significant portion of participants. The study revealed that 1 in 3 participants did not wash their hands after touching raw chicken and before touching a smart device. Additionally, 74% did not wash their hands after handling raw eggs and before handling their smart device.

The Microbial Terrain:

Microbial analysis further illuminated the potential risks lurking on our smart devices. Food poisoning bacteria such as *Salmonella* and *E.coli* were found to survive on smart device screens for more than 24 hours. During a 30-minute cooking activity, participants touched their smart device on average almost six times. *Enterobacteriaceae* contamination was detected on 6% of pre-cleaned tablets, showing cross-contamination of these devices during the cooking activity.

Insights from Consumer Perspectives:

Delving into consumer perceptions through focus groups and an online survey uncovered nuanced attitudes towards food safety and smart device usage. While participants acknowledged the risk of bacterial transfer from devices, there existed a low perceived risk of contracting food poisoning at home. Moreover, socio-demographic factors such as gender and education level influenced both self-reported and observed behaviours, indicating varying levels of attentiveness to hygiene practices.

Implications and Recommendations:

The findings of the research project have implications for both public health and consumer education. Despite the widespread adoption of smart devices in the kitchen, there exists a critical need to enhance consumer awareness of the associated food safety risks. This is particularly relevant for those preparing food for people in vulnerable groups such as young children, those over 65, pregnant individuals or those with an underlying medical condition which compromises their immunity.

Recommendations from the project included promoting regular disinfection of smart devices and advocating for a dedicated kitchen device to mitigate the risk of cross-contamination. Using antibacterial wipes containing alcohol can significantly reduce the contamination on smart device surfaces. In addition, encouraging consumers to adopt simple yet effective measures, such as handwashing between handling raw ingredients and touching devices, we can collectively strive towards safer kitchen environments.

Dr. Mairead McCann, Technical Executive at safefood, emphasised the importance of this research, stating, "Understanding the potential risks associated with using smart devices in the kitchen while cooking high-risk foods is crucial for ensuring food safety in our homes." This sentiment underscores the necessity of addressing these findings to safeguard public health.

safefood's Top Tips for Good Food Safety Habits While Using a Smart Devices in the Kitchen:

Wash hands before and after cooking:

Clean hands are important to help stop cross-contamination between your smart device and ingredients when cooking. Before and between handling ingredients and your smart device, wash your hands thoroughly with warm water and soap and drying with a clean hand towel.

Clean worktops before and after cooking with a smart device:

When using a smart device while cooking, placing your device on an unclean kitchen worktop could spread harmful bacteria. Always wash kitchen worktops with hot soapy water before and after preparing food. Cleaning as you go will help reduce cross-contamination and keep your workspace clear.

Disinfect your smart device before and after cooking:

Before you start cooking, disinfect your smart device to help reduce potential cross-contamination. It's important to also disinfect your smart device after cooking if you have handled raw ingredients such as meat, poultry or eggs. Always follow your manufacturers' instructions before doing so. Using antibacterial wipes (containing at least 70% alcohol) can significantly reduce the contamination on smart device surfaces. When disinfecting, pay close attention to the screen, buttons and edges.

Conclusions:

While smart devices have transformed the way we approach cooking and meal preparation, their integration into the kitchen presents its own set of challenges. By combining technological convenience with an awareness of food safety, we can keep our cooking both creative and safe for everyone.

References:

[1]https://www.safefood.net/professional/research/research-reports/smart-devices-in-the-kitchen (Accessed 05/07/24)

Locating homicide victim deposition sites associated with souterrains in the Republic of Ireland in 2023

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Keywords: Souterrain, Ireland, Forensic, Clandestine Burial, Homicide

Introduction:

Souterrains (an underground passage or chamber) are a significant, well-known part of Irish history, built within and around other historic monuments (Dowd et al 2017). A souterrain is a historical landmark most widely recognised in early medieval monuments and in early Christian ecclesiastical sites in Ireland with over 3,500 recorded (Clinton, 2001; Dowd, et al., 2017; Manning, 1998). They are a manmade architectural room or passageway created underground (like a trench) often near or within a ringfort and are often made from timber, stone or both. Structurally they are often a passage and/or a chamber.

Irish souterrains were constructed either by tunnelling in clay or rock or by drystone or timber construction in a subsequently backfilled trench and though they vary significantly in plan and range in length from 5m to 100m. They are invariably composed of a number of repetitive elements and have a date-range from AD c. 600 to 1200.

Historically they were used for cold storage and refuge but it has been recently proposed that the stable temperature in a souterrain was particularly suited to the storage of dairy products and it was suggested by Estyn Evans in 'Irish Folk Ways', that the butter deposition in bogs may have started due to souterrians not being used (Synott and Sikora, 2019). A souterrain recorded on a reported on historic landmarks in the county of Sligo document, was in Cooldrumman Upper Townland, Sligo, Republic of Ireland and the most recent documented use of this souterrain was by the Irish Republican Army (IRA) during the War of Independence.

These semi-hidden underground environments have always had the potential to be criminalised and although many have been located and documented, there are many more that are not. They therefore offer the opportunity to be used to store drugs, ballistic weapons, ammunition, homicide victims and potentially missing persons (Harrison and Donnelly, 2019; Donnelly and Harrison, 2020). The "Missing Person Statistics" in Ireland as of 2022 (An Garda Siochana, 2023). suggested that there were a total of 10,512 missing person reports with most of them resolved, however there were 61 persons still missing as of October 2023.

There are many reasons that these people may not have been found such as immigration or death however many people are missing due to unknown causes such as the 'disappeared'. The disappeared consist of 17 people who went missing over the course of the troubles in Northern Ireland, fortunately 13 of these people have had their remains recovered however 4 remain missing. The disappeared are not the only people who may benefit from this research on souterrains as there are multiple missing persons across Ireland with many in rural areas. There are souterrains all over the country and many of them have suffered natural overgrowth from the field that they are in and are then unnoticeable to the those who don't know they are there. Many souterrains are within fields and are unmarked and in some cases they have been covered by cattle grids due to looking like holes in grassland but they may be a lot deeper and unstable with the potential of objects (or people) lost in them.

Locating unknown souterrains can be difficult, they may be on private land, or may simply be well hidden by the detritus of time and the elements. If identified for nefarious reasons, they offer a challenge to Police services in locating them.

The first statement of the 2nd Law of Thermodynamics directs that heat flows spontaneously from a hot to a cold body – it tells us that an ice cube must melt on a hot day rather than becoming colder. To assist location of such concealed environments, the possibility that temperature differentials between the souterrain interior and the outside may be used was experimentally tested as the implication is that on hot sunny day, the interior would initially be colder from a cool night previously but would warm-up during the day. Equally on a cold day outside, the interior of the souterrain would potentially be warmer by some degrees but would cool down over the daytime. Being able to identify this difference using thermal imaging would offer a fast non-invasive method of location, perhaps in combination with forensic detection dog scenting and drone investigations for terrain anomalies (DesMarais 2014; Mires 2019;, Pensieri et al,2020; Kumar, 2022; Ferguson and Gaub, 2023).

This was done by conducting two experiments running concurrently. An experimental manmade souterrain was constructed to begin developing a valid model for future investigation testing the temperature and humidity parameters inside the structure and comparing them with the outside environment temperature and humidity Vapour Pressure Deficit (VPD) and Dew Point (DP) were also monitored. The construction of a souterrain was conducted on the 13th of February 2023, on an area of land owned by Atlantic Technological University Sligo as within this area there was a previously built ringfort by the archaeology department who gave their permission for it to be used. This ringfort had a hole in the centre which needed to be cleared and levelled for the structure to be built involving a plywood frame using nails and a hammer to hold it in place over the cardboard box.

The box and frame were then placed in the hole with the entrance facing east, to absorb the maximum heat and sunlight throughout the day, then the tiles were secured by using an outdoor sealant to hold them against the plywood while the dirt/mud, that had been dug out from levelling the hole, was stacked against the tiles. Grass was then placed on the roof of the souterrain to keep it hidden from potential onlookers outside the field. After the structure was secured in place a small device was placed inside, the Govee indoor thermometer H5075, this device was then used to measure the temperature, humidity, DP and VPD within the souterrain. The temperature and humidity of the environment surrounding were measured using a weather app on a Samsung tablet device, which via Bluetooth connected to the H5075 device to download the data from inside the souterrain. Data was collected following the souterrains completion at 4:30 pm every day from the 13th of February 2023 to the 31st of March 2023 in order to obtain the most accurate readings and thermal images were taken with the FLIR bXX series iXX series and FLIR ix series Extech IRC30.

Secondly a publicly accessible historic souterrain in Cashelgarran [Figure 1] (https://mapcarta.com/18968202 geographical location: Sligo, North West, Ireland, Europe, geographical coordinates: 54° 21' 48" North, 8° 31' 23" West) was investigated as it offered a much larger, longer standing structure to compare with the smaller experimental model built on the University grounds (Latitude: 54° 16' 40.80" N, Longitude: -8° 27' 36.00" W), Figures 2-4.

Over a period of a few months (January-March 2023) measurements were recorded of temperature and humidity and Vapour Pressure Deficit and Dew Point. Outside temperature was monitored using local metrological data. Internal souterrain temperature in the experimental site was obtained using a Bluetooth 'Govee H7075 Indoor Thermometer'. Forward Looking InfraRed Infrared - FLIR thermal imaging cameras were employed; FLIR bXX series iXX series and a FLIR ix series Extech IRC30. (https://www.flir.co.uk/)

Results:

The temperature data collected from the H7075 device over the period January to March, and the local weather website show a comparable trend throughout the weeks the data was collected as seen in graph 1. The graph shows that the inside of the manmade souterrain remains cooler than the outside environment for the majority of the time and it can be seen that it changes with the change in weather while remaining cooler.

Figure 2

Figure 1

Figure 3

Figure 4

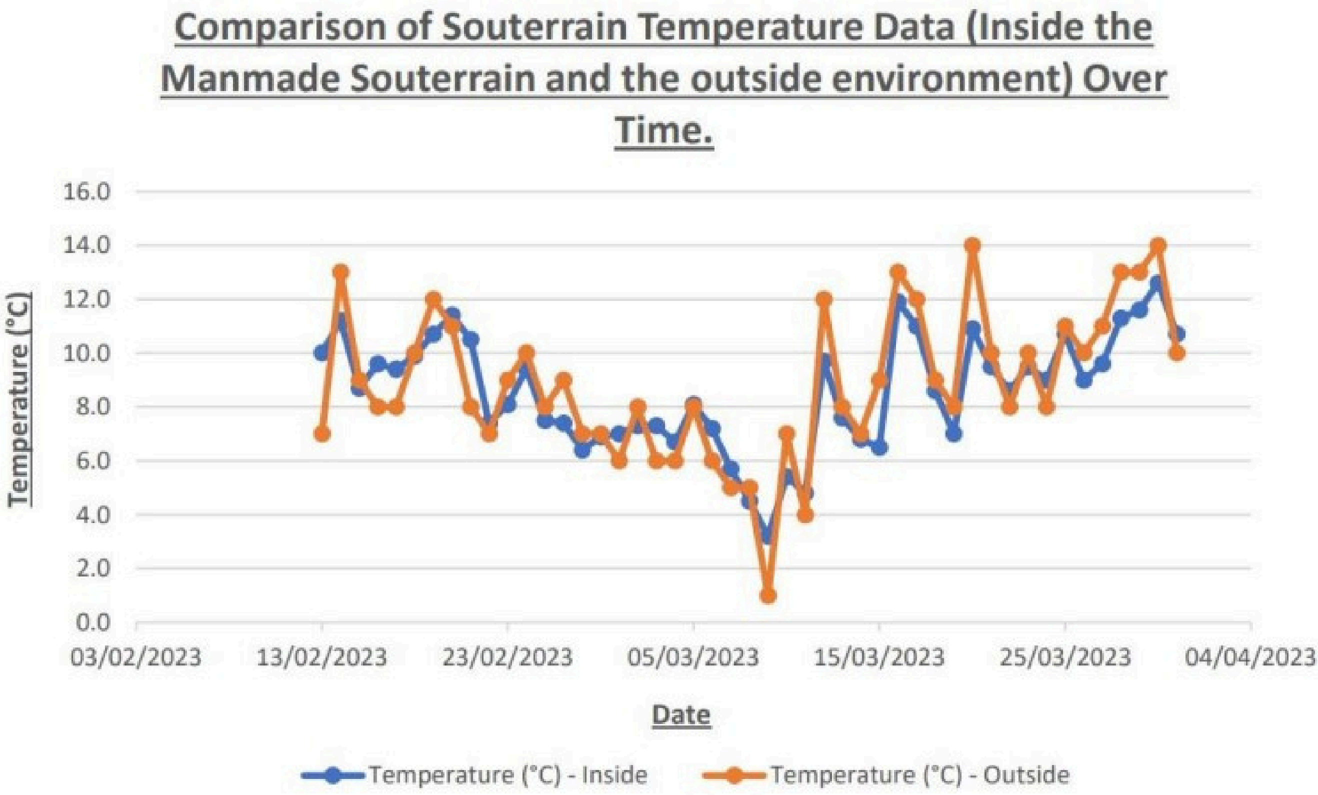

Figure 5

Figure 6

There is a minimum of 0.5 °C between each data point collected apart from one data set as there was a drop in temperature on the 9th of March which can be seen in the graph, The weather consisted of strong winds, snow showers and icy weather contributing to the outside temperature being lower than the inside temperature. As the outside temperature proceeded to increase the inside temperature remained cooler indicating that when the temperatures outside the souterrain are warm then the inside of the souterrain is cooler than the outside temperature however, when the temperature drops drastically outside then the temperature within the souterrain is warmer.

The graphs of temperature data (Figure 5) demonstrated that the temperature outside and inside the experimental souterrain aligned closely, but there was a difference of a few degrees Celsius, which was within the detectable parameters of the basic thermal imaging cameras and would certainly be within higher specification thermal imaging devices. Thermal imaging studies at the historic souterrain in Cashelgarran showed that, in principle, a visible difference between the internal and external temperatures was detectable but it identified that further work and more suitable souterrains are sought which have smaller openings and the ability to temporarily close the entrance.

Conclusions:

There is currently a toolkit of complimentary methods for the location of potential clandestine burial sites. This toolkit is necessary as not all techniques will be appropriate for all sites due to the complexity of the environment etc. The development of new technologies particularly non-invasive ones that can be brought to bear on such investigations is important to further assist Police investigations. Therefore, the concept of the thermodynamic principle (cool air and condensation) associated with souterrains ubiquitously found in Ireland, offers a novel method of remote detection of these Iron Age underground cellars using temperature variation from the external environment.

The data presented in this study shows the experimental-manmade souterrain retains cool/hot temperatures, depending upon the external temperature. The humidity, dew point, and how different they are from the outside temperatures and humidities also offer potential parameters to detect differences and hence identify existing souterrains clandestine locations. This data may help in the detection of souterrains as a drop in temperature may indicate the entrance or opening, even if they are collapsed stone structures. The data presented in this study will serve as a basis to conduct further work from a concept to a practical methodology for police searches.

This study considers that souterrains, which may currently be used for the deposition of homicide victims has to be carefully selected by perpetrators in which specific parameters are sought. This offers further discriminating inclusion/exclusion criteria for police when searching areas of interest (e.g., isolation from population, distance from roads, out of human memory if previously located etc.). The results from this data may facilitate advances in body recovery in geographies such as Ireland and has the potential to assist in the resolution of homicide cold cases.

Acknowledgments:

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References:

[1] Clinton M, 2001 The Souterrains of Ireland, Wordwell, 1869857496

[2] DesMarais AM. Detection of Cadaveric Remains by Thermal Imaging Camera, Journal of Forensic Identification 2014;64 (5):489.

[3] Dowd Moore S, Sullivan I and Sullivan S. 2017. Reinventing the Souterrain, Archaeology Ireland, 2017;31:26-30.

[4] Donnelly and Harrison M. The Geoforensic search strategy: A high Assurance Search Method to Assist Law Enforcement Locate Graves and Contraband Associated with Homicide, Counter Terrorism and Serious and Organised Crime. J. Geol. Soc. Japan, 2020;126 (8): 443–458,

[5] Ferguson and Gaub JE, Training Police Search and Rescue Teams: Implications for Missing Persons Work. Criminology and Criminal Justice, 2023;23(3):431-449. https://doi.org/10.1177/17488958211057380

[6] Harrison M, Donnelly L. 2019 The Geoforensic Search Strategy: A High Assurance Search Method to Assist Law Enforcement Locate Graves and Contraband Associated with Homicide, Counter Terrorism and Serious and Organised Crime, Burnley, Lancashire: IUGS Initiative on Forensic Geology

[7] Keatley D, O'Donnell C, Chapman B and Clarke DD. 2022. The Psycho-criminology of Burial Sites: Developing the Winthropping Method for Locating Clandestine Burial Sites. Journal of Police and Criminal Psychology, 2022;37: 91-100

[8] Kumar A. (2022). The Role of Dogs in Search and Rescue. In: Verma, S., Prem, H.T. (eds) Management of Animals in Disasters. Springer, Singapore. https://doi.org/10.1007/978-981-16-9392-2_16

[9] Mannin C, 1985, Irish Field Monuments 1998, Office of Public Works,Ireland, 978-0707600352

[10] Mires AM. (2019). The Use of Forensic Archaeology in Missing Person Cases. In: Moran, K.S., Gold, C.L. (eds) Forensic Archaeology. Springer, Cham. https://doi.org/10.1007/978-3-030-03291-3_8

[10] Pensieri MG, Garau M, Barone PM. Drones as an Integral Part of Remote Sensing Technologies to Help Missing People. Drones 2020,;4,-15. https://doi.org/10.3390/drones4020015

[11] Pringle J and Ruffell A. (2017). The Science of Finding Buried Bodies. The Conversation, 7783. https://theconversation.com/the-science-of-finding-buried-bodies-77803

[12] Synott C and Sikora M. 2019. New Dates for Old Butter. Archaeology Ireland, 2019;32(125); 26 - 29

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Academic Programmes

Bachelor of Science Forensic Investigation and Analysis at the Department of Life Sciences. School of Science. Atlantic Technological University Sligo, Ireland. The programme encompasses the application of a forensic investigative approach using advanced analytical science for the provision of scientific data and evidence. The underlying analytical science combines forensic, biological, chemical, communication and information technology skills. These skills can be applied to the investigation of crime, testing for toxins or illicit drugs, DNA profiling or statistical analysis.

Programme Overview:
This 4-year programme gives graduates skills in both forensic and analytical science making them highly employable in a broad range of sectors. Students will study both biology and chemistry through the exciting and stimulating medium of forensic science. A major focus of the programme is the development of excellent practical analytical science skills which are in great demand by employers and for postgraduate research. The stimulating programme facilitates engagement with a variety of learning experiences including the following :

- simulated crime scenes with practicing forensic investigators
- training in molecular biology techniques for the development of DNA profiles
- collection and chemical analysis of gunshot residue
- learning how to test for toxins and illicit drugs
- engagement in flexible student centred work experience
- expert witness training and activities to enhance communication skills
- projects involving information technology and advanced scientific instrumentation.

Year One:
Students are provided with a solid foundation in Biology, Chemistry, Physics and Mathematics, as well as introductory modules in Information Technologies, Criminal Justice and Forensic Science.

Year Two and Three:
First-year modules are studied more in-depth as the programme progresses with added subjects such as Crime Scene Investigation and Management and Instrumentation used for Forensic Analysis, Genetics, Molecular Biology, Statistics and Quality Assurance

Year Four:
This final year focuses on high-level investigative, observational, evidence interpretation, research and crime scene management skills. Students will complete their work experience, presentation and forensic based research project in this year. Graduates from this course will be versatile with key skills in chemical analysis, bio-analysis, information technology and communications, and project management. These will enable them to attain employment in laboratories in a variety of sectors from forensics, environmental, pharmaceutical and food industries as well as engaging in further postgraduate studies.

Professional Accreditation:
This programme is accredited by the Chartered Society of Forensic Science in the United Kingdom for the component standards Interpretation, Evaluation and Presentation of Evidence (IEPE), Crime Scene Investigation (CSI) and Laboratory Analysis (LA). As the first third-level course on the island of Ireland to achieve this accreditation, it gives graduates the assurance that they have an internationally recognised qualification and are ready to undertake a professional career in forensic science. This programme is also aligned to the Teaching Council of Ireland guidelines for secondary school teaching of science and chemistry. To become a fully qualified secondary school teacher, students need to complete a Professional Masters in Education (PME) after they graduate.

For more information please see https://www.itsligo.ie/courses/bsc-hons-in-forensic-investigation-and-analysis/ (Accessed 03/06/24)

Master of Science in Forensic Medical Sciences at the Cameron Forensic Medical Sciences Centre for Clinical Pharmacology and Precision Medicine, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, United Kingdom.
An unprecedented programme offered by Cameron Forensic Medical Sciences right where Sherlock Holmes and Dr John Watson first met and conducted experiments to solve crimes and only a few hundred yards away from the Jack the Ripper murder sites, covers a wide range of specialist topics under the umbrella of the forensic medical sciences, coupled with the opportunity to carry out research in a specialist area.

In the module "Forensic Pathology" and in module "Legal and Ethical Issues in Forensic Medical Sciences," students study forensic pathology and visit London mortuaries to observe autopsies, attend court hearings gaining knowledge of how injuries are interpreted and how cases are prepared for court. In module "Clinical Forensic Medicine" students study the role of the doctor in assessing persons in custody, assault victims, child maltreatment and assessing torture victims, etc.

In the module "Forensic Toxicology" students study alcohol and drugs and their misuse and how these substances are screened for, detected, quantified and interpreted in forensic medico-legal cases.

In the module "Forensic Human Identification" students study the various methods by which deceased and living persons can be identified both as individual cases and in mass disasters, including by DNA, dental and other methods.

Please see More Information: https://www. qmul.ac.uk/whri/cameronqmul (Accessed on: 23/06/24)

Academic Programmes

Master of Science (MSc) in Analytical Toxicology at the Faculty of Life Sciences and Medicine, Kings College London is a unique study course that integrates theoretical and practical aspects of analytical science with clinical and forensic toxicology.
Designed for scientists wishing to enter the field of clinical or forensic toxicology, or for clinical and forensic practitioners who want to develop their existing knowledge and professional experience.

Key benefits:
• The programme is run by the King's Forensics team (also responsible for the MSc Forensic Science) whom are at the forefront of research in analytical techniques in both forensic science and toxicology/drug analysis. King's Forensics also has two ISO 17025 accredited laboratories (drugs and DNA).
• Combining theory and practical work, this programme has been developed with the collaboration of both national and international experts in the field of clinical and forensic toxicology.

Entry criteria:
• Minimum 2.1 degree or an overseas equivalent in chemistry, biochemistry, pharmacy, forensic science or related discipline
• English language band: D

The course will be delivered by lecture with lecture recordings available to allow for flexibility of learning styles. There will also be laboratory practical components, workshops, group discussions and problem- based learning exercises.
The approximate total contact hours for the taught modules are 390 hours. You are also expected to undertake approximately 810 hours of team and individual study.
Taught modules total 120 credits. The analytical toxicology research project module is worth 60 credits. Project selection is through student application and interview.
Typically, one credit equates to 10 hours of work.

For more information please see

kcl.ac.uk/study/ postgraduate-taught/courses/ analyticaltoxicology-msc (Accessed 05062024)

This section on academic programmes in our professional magazine was prepared in June 2024. Although it was up-to-date at the time it was produced, please make sure you check the course websites directly for the very latest information before you commit yourself to any of the courses.

Acknowledgements

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Arabic • Chinese • English • French • Greek • Hindi • Portuguese • Spanish • Turkish

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Selected Graphics from Previous Meetings

AUTUMN 2020 VIRTUAL SCIENTIFIC MEETING

Scientific Program

MONDAY, 02-NOV-2020

(Listed Times Reflect London, UK Time)

14:00-14:15	Welcome and Introduction Nikolas P. LEMOS - IACFT
14:15-15:00 (PL01)	Forensic Toxicology and Drug Control in a Post-COVID-19 World Justice TETTEY
15:00-15:15	Q & A with the Speaker via Chat
15:15-15:30 (M1)	Assessment of alcohol consumption during COVID-19 pandemic by means of ethyl glucuronide concentrations in hair samples of selected populations Lia VISINTIN
15:30-15:45 (M2)	Abused substances during the COVID-19 pandemic in Izmir, Turkey Melike AYDOĞDU
15:45-16:30 (PL02)	Strengthening the global approach to postmortem toxicology interpretation Bronwen DAVIES
16:30-16:45	Q & A with the Speaker via Chat
16:45-17:00 (SP01)	Sponsored Presentation Finden by KURA biotech
17:00-17:15	BREAK
17:15-17:30 (M3)	New trends in drug diversion and drug use in the western region of Algeria Atika BENDJAMAA (SATOX PRESENTATION)
17:30-17:45 (M4)	CBD - The Swiss legislation accelerating CBD to become a pharmaceutical drug Wolfgang WEINMANN

(CONT.)

Online Resources

Archived on demand selected meetings available at the Center for Forensic Science Research & Education (cfsre) website

Go to https://forensiceducation.Brightspace.com/d2l/home

Online Resource

New Website for IACFT Gazette

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Special Issue Notification Section

IACFT would welcome proposals for new special issue topics

Current open call for papers in the journal Frontiers in Molecular Biosciences

Current Trends in Targeted and Non-Targeted Metabolomics in Analytical Toxicology

Topic Editors

Geraldine M. Dowling SFHEA, Markus R. Meyer

Frontiers in Molecular Biosciences
Metabolomics

Topic Published	Manuscript Summary Deadline	Manuscript Deadline	Extended Deadline
22/03/2023	28/08/2023	29/11/2024	30/11/2024