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International Alliance of Clinical and Forensic Toxicologists

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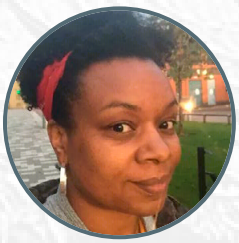
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IACFT Journal Editor-in-Chief
Dr Geraldine M. Dowling SFHEA

Message from the Editor-in-Chief

There is much to celebrate at the International Alliance of Clinical and Forensic Toxicologists (IACFT) and it is both an honour and a pleasure to present the third volume of our journal- now officially renamed as the IACFT Journal, with a new design and an assigned ISSN number.

This newly rebranded edition features peer-reviewed articles and curated content selected by our editorial team to engage, inform and inspire our global readership. We are especially pleased to announce the expansion of our content through the introduction of the "Science in Motion" section, which will include case studies and contributions from disciplines beyond traditional IACFT fields, further enriching the journal's scope and relevance.

The IACFT is a global consortium of scientists dedicated to advancing forensic and clinical toxicology through research and its application in service to local communities. Recognising the constraints of time and budget that often limit participation in international conferences, IACFT continues to foster meaningful collaboration and impactful contributions to the field grounded in rigorous empirical research and high-quality data.
We are confident that those who have attended IACFT's online meetings or engaged with previous journal editions have found great value in the scientific presentations and lively informed discussions.
All editions of the IACFT Journal, along with our other online educational resources, are freely available to support accessible knowledge-sharing across borders.

We extend our sincere appreciation to our meeting organisers, speakers, sponsors and editorial contributors, whose dedication has ensured the smooth delivery of our events and publications before, during and following the challenges of the global pandemic. Thanks to these efforts, our virtual meetings have welcomed participants from over 65 countries, greatly expanding global access to forensic and clinical toxicology discourse.
In light of these accomplishments and in celebration of the vibrant community we serve, we are proud to launch the redesigned IACFT Journal. This publication is intended for professionals, academics, researchers and students engaged in clinical and forensic toxicology. Our editorial team, led by myself as Editor-in-Chief, along with sub-editors and our partners from industry and academia, represents a diverse and inclusive community. We remain steadfast in our commitment to fostering a welcoming environment where all contributors and readers feel respected, valued and heard.

Moving forward, inclusion and diversity will continue to be central to our work in research, education and service delivery. As a digital-only publication, the IACFT Journal embraces innovative formats, allowing for the integration of multimedia elements such as audio, video and interactive hyperlinks to enhance the reader experience.
Content from IACFT meetings on the Centre for Forensic Science Research and Education (CSFRE) website is recognized for continuing professional development by the American Board of Forensic Toxicology (ABFT) and the American Board of Criminalistics (ABC) enhancing professional practice worldwide.
Archived on-demand meetings are available at: forensiceducation.brightspace.com/d2l/home
Please note, these resources are freely accessible.

We hope you enjoy this third volume of the IACFT Journal and we welcome your feedback and suggestions.

From the very beginning of the IACFT publishing journey, my dog Lexi was by my side as a loyal companion through every step. Sadly, she passed away in September 2025 and I dedicate this volume to her memory.

Lexi
22/03/2014 – 04/09/2025
Forever missed

Guidelines for IACFT Authors

The IACFT Journal welcome articles from the readership. The format for the IACFT Journal articles must follow the following guidelines.

Artwork/Pictures:
The artwork if present should be sent in a separate file. The artwork files should be in an acceptable format (JPEG or high quality PDF).

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Academic Partners, Funding Support and Accessibility

There is much to celebrate at the International Alliance of Clinical and Forensic Toxicologists (IACFT) and we are both delighted and honoured to present the third volume of the IACFT Journal and the launch of its new design.

This project was supported through funding from the Irish National Forum for Teaching and Learning, under the Strategic Alignment for Teaching, Learning and Assessment (SATLE) initiative. It was undertaken in 2022 as a Learning Enhancement Project at Atlantic Technological University, Sligo (formerly Institute of Technology Sligo), through the Teaching and Learning Centre.

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. Funding to support the redesign for Volume 3 of the IACFT Journal was facilitated by the ATU Library.

The support secured was utilised to help undergraduate students, IACFT professionals, academics, researchers, postdoctoral fellows and postgraduate students share or create learning resources.

In addition, IACFT aims to publish interesting, innovative, unusual and otherwise noteworthy peer-reviewed material in the IACFT Journal, as well as to develop alternative continuing professional development (CPD) learning resources using Universal Design for Learning (UDL) and Community-Based Learning (CBL) pedagogies. These approaches facilitate the sharing of information in multiple formats for students and professionals, making IACFT resources accessible to individuals with non-traditional learning preferences and to learners at all stages of their toxicology careers.

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.

IACFT acknowledges its academic and educational partners for their support of the rigorous peer-review processes and editorial practices that underpin this journal. Their contribution ensures the journal serves as a platform for new research in the field while enhancing toxicology CPD opportunities through an open-access publication accessible to a global audience.

Ollscoil Teicneolaíochta an Atlantaigh

Atlantic Technological University

Editorial

IACFT Journal Volume 3 December 2025

Geraldine M. Dowling [1-4]

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It is my pleasure to welcome readers to Volume 3 of the IACFT Journal, which continues to reflect the journal's mission to provide a rigorous, inclusive and globally relevant platform for scholarship in forensic and clinical toxicology, as well as a continuing professional development (CPD) resource. My role in shaping and leading this editorial direction reflects strategic academic leadership in supporting disciplinary CPD scholarly standards and inclusive educational practice within forensic and clinical toxicology.

The awarding of an ISSN and the introduction of a new journal design represent important milestones in the journal's development. Key indicators of the journal's sustained growth and impact include:

- The granting of an ISSN (3088-6600) by the National Library of Ireland (2025), affirming the journal's scholarly legitimacy.
- The journal has expanded from six peer-reviewed papers from institutions across five countries in 2023, to fourteen papers from institutions across nine countries in 2024 and fifteen papers from institutions across nine countries in Volume 3 in 2025. These articles were all authored by undergraduates, postgraduates, clinical and forensic practitioners.

Together, these developments reflect sustained academic stewardship, robust quality assurance processes and the consolidation of an international scholarly community of practice supporting education, research and CPD in toxicology.

As part of our sustainability strategy to ensure the IACFT Journal remains a free and accessible CPD resource for all, we are progressing plans to partner with a forthcoming Atlantic Technological University (ATU) co-led national pilot platform for fully open-access journals in Ireland, anticipated to launch in early 2026. This initiative, involving higher education institutions across Ireland, is funded by the Higher Education Authority (HEA) through the National Open Research Forum (NORF). This work demonstrates leadership beyond the institution, contributing to national conversations on open research, equitable access to knowledge and the sustainability of CPD-focused scholarly publishing.

The IACFT Journal publishes noteworthy peer-reviewed content and develops CPD resources in clinical and forensic toxicology, guided by evidence-based pedagogical principles such as Universal Design for Learning (UDL) for creating content and Community-Based Learning (CBL). These approaches are intentionally used to enhance accessibility, support learner autonomy and maintain professional relevance for diverse learners and practitioners at every career stage, reflecting a commitment to inclusive curriculum and CPD design. This volume showcases the breadth and diversity of contemporary forensic and clinical toxicology teaching informed by UDL and CBL alongside related research and practice. Contributions span analytical innovation, interpretative frameworks, public health perspectives and professional development across the career lifespan.

A defining feature of this volume is its strong international focus, with contributions from Europe, Africa, Oceania and South America highlighting both region-specific challenges and globally relevant developments. This international engagement enriches CPD by exposing learners and practitioners to diverse regulatory, cultural and professional practice contexts. The article by Menéndez-Quintanal et al., entitled *"New Psychoactive Substances and Chemsex: A European and Spanish Overview, with Clinical and Forensic Case Studies from the Canary Islands"*, provides a timely examination of emerging drug trends and their clinical and forensic implications. Similarly, the article by Ayisat Aneyo et al., *"Nigeria's Drug Crisis and Youth Vulnerability: An African Perspective on Local Concoctions"*, offers an important public health perspective, emphasising sociocultural context and harm-reduction approaches.

Analytical and methodological advances are well represented in this volume. Articles such as *"First Analytical Identification of N,N-Dimethylpentylone in Argentina: Analysis of a Seized Sample" by Zar et al., "Dried Matrix Microsampling for a Reliable Approach to Workplace Drug Testing"* by Galletto and *"Cell Viability Assays for Use in Determination of Toxicology"* by O'Dowd demonstrate the continued evolution of tools and techniques available to forensic and analytical toxicologists, supporting evidence-informed professional practice and CPD.

Another focus of IACFT is to encourage undergraduate and postgraduate students, through UDL and CBL pedagogies, to contribute to their own CPD by creating learning resources for the forensic and clinical toxicology community. This approach supports students as emerging professionals and contributors to disciplinary CPD, demonstrating leadership in mentoring, capacity-building and the development of scholarly and professional identity. Forward-looking student perspectives are explored by Klorek et al. in *"The Potential of Aptamers in Analytical Toxicology"* and by Alsumain et al. in the commentary "Is Metabolomics a Useful Tool for the Evaluation of the Emerging Group of 'Nitazene' 2-Benzylbenzimidazole Synthetic Opioids?", both of which encourage critical engagement with emerging technologies. In addition, McQuillan et al., *"Combining Genetic Analysis with Forensic Toxicology to Distinguish Between Accidental and Intentional Overdoses",* introduces challenges increasingly encountered in contemporary practice.

Interpretative and case-based scholarship remains a core strength of the journal, exemplified by Martínez et al. in *"Criteria for Interpretation of Postmortem Toxicological Results"*, as well as by the chemsex case-study interpretations featured in this volume. The clinical relevance of toxicology is further illustrated by Jiranantakan in *"Lidocaine Toxicity Following Tumescent Liposuction"*, underscoring the importance of interdisciplinary collaboration between clinical and forensic domains.

This volume further reinforces the journal's commitment to education, training and professional development dedicating articles to the Science in Motion section, which invites studies from disciplines beyond toxicology. By foregrounding interdisciplinary and experiential learning, this section models innovative approaches to professional education and CPD beyond traditional disciplinary boundaries. Coyne et al.'s study, *"Person-Centred Practice in Action: A Case Study from Practice Placement Learning"*, highlights experiential learning, while Vasconcelos's study, "Drug Use and Misuse Trends in Older Adults Since 2010", addresses an often under-represented population in toxicology education resources and research. The Career Spotlight features on Associate Professor Ghadeer Abdelaal and Dr Rachel Marr celebrate diverse career pathways and aim to inspire both early-career and established professionals.

In *From Ligands to Botanical Complexities: The Analytical and Clinical Evolution of the Australian Poisonous Plants Project*, Caldicott observes that, at first glance, a crushed tablet on a nightclub floor and a leaf from the Australian bush appear to have little in common. One is rooted in synthetic chemistry and the practice of nightlife harm reduction; the other in botanical taxonomy and rural toxicology. Yet both ultimately converge at the intersection of analytical chemistry, clinical medicine and public health communication. The transition from frontline drug checking ("pill testing") to the revitalisation of the Australian Poisonous Plants Project (APPP, established in 1984) is therefore a logical progression, reflecting broader shifts in how we identify, analyse and respond to chemical threats - whether manufactured or naturally occurring.

IACFT remains committed to reflective enhancement of open-access publishing and inclusive educational practices that support lifelong learning and CPD across clinical and forensic toxicology. These commitments continue to inform editorial strategy, peer-review processes and the future development of the journal. I would like to acknowledge our academic and educational partners for their ongoing support of the rigorous editorial and peer-review standards that underpin this work.

On behalf of the IACFT, I extend my sincere thanks to the authors, reviewers and readers whose engagement sustains the journal as a collaborative international community of practice. I hope this volume informs, challenges and inspires further education, research in UDL and CBL, digitally enabled learning and professional practice across the global forensic and clinical toxicology community, while contributing to the ongoing enhancement of CPD, educational practice and disciplinary standards.

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New Psychoactive Substances and Chemsex: A European and Spanish Overview, with Clinical and Forensic Case Studies from the Canary Islands

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Keywords: Chemsex, New Psychoactive Substances, Cathinones, 2-fluoro-deschloroketamine (2F-DCK), N-ethyl-deschloroketamine (2-oxo-PCE), High Resolution Mass Spectrometry.

1. Introduction to Chemsex

European Perspective and the Spanish context

The term “Chemsex”, originating in the UK, comes from the combination of the words “chems” (short for “chemicals”, referring to drugs) and “sex”. It describes a specific type of sexualized drug use, primarily among men who have sex with men (MSM). Chemsex is characterized by the consumption of drugs to enhance sexual experiences, often resulting in prolonged sexual sessions that can last for hours or even several days. This phenomenon is also known as "party and play" in the United States and as "intensive sex partying" in Australia. Chemsex sessions can involve various encounters, including one-on-one interactions with casual or steady partners, threesomes or group sex. Some individuals, however, may also engage in Chemsex alone, often watching pornographic material or interacting online with other participants through webcams [1-4]. Typically, Chemsex sessions are held in private homes, but they can also take place in gay-oriented venues such as saunas, sex clubs, hotels, private gatherings, dark room spaces, festivals designed for sexual activity and outdoor cruising spots.

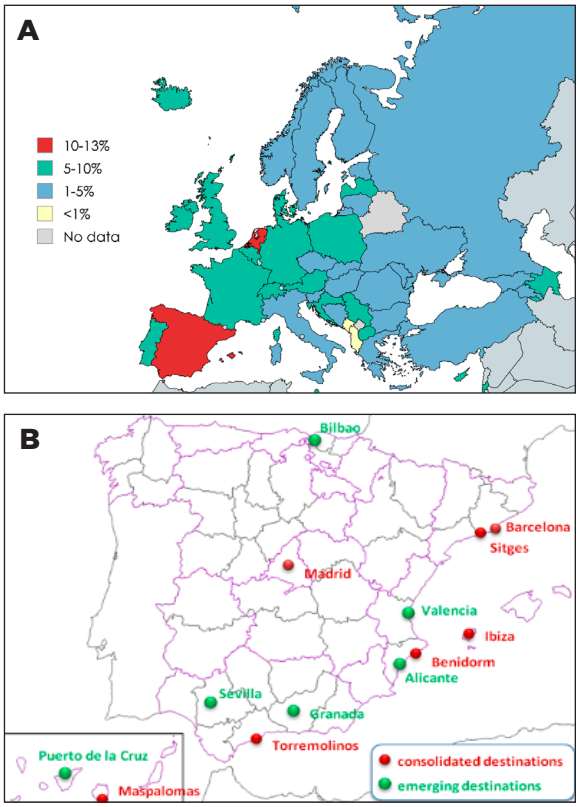
Chemsex is commonly associated with mephedrone (4-methylmethcathinone), methamphetamine and gamma-hydroxybutyric acid/gamma-butyrolactone (GHB/GBL) [5]. These substances, originally called "chems," [1] are known for their ability to induce sexual disinhibition and excitement—effects that are not typically associated with drugs like ectasy (MDMA). While the initial definition of Chemsex focused on these three substances, the use of other drugs or combinations—including cocaine, amphetamines, MDMA, nitrite inhalants (poppers), ketamine and phosphodiesterase-5 (PDE5) inhibitors for erectile enhancement—varies depending on factors such as availability and geography [4]. With the emergence of new psychoactive substances (NPS) in the illegal market, additional compounds—such as cathinone derivatives, phenylethylamines and ketamine analogues—are increasingly incorporated into Chemsex practices. This expansion makes the range of substances involved in Chemsex broader and less well understood.

Polydrug use is frequent during Chemsex sessions, with diverse administration methods including oral, nasal, inhaled, smoked, rectal, intravenous and intramuscular. Injected drug use, known as "slamming," is less common but carries the highest health risks. When taken in a sexual context, these substances can induce intense arousal, euphoria and a sense of disinhibition. This state can lead to difficulties in setting boundaries, refusing sexual

partners or leaving the sexual session. In certain situations, this may lead to dynamics that promote high-risk behaviours including both sexual and drug-related activities, as well as extreme sexual practices. Chemsex is predominantly an urban phenomenon, making it more prevalent in large cities where there are significant gay communities. It is also common in popular gay tourist destinations.

From a European perspective, the European Men-who-have-sex-with-men (MSM) Internet Survey (EMIS)—a multi-country, multi-lingual, and anonymous online survey of gay, bisexual, and other MSM—was conducted across 50 countries in 2024. Preliminary findings from EMIS-2024 [6], based on 50330 completed surveys, indicate that participants were asked whether they had engaged in chemsex during the previous four weeks. The highest prevalence was reported in the Netherlands (12%), Spain (12%) and Belgium (10%). Overall, chemsex was more common among respondents living with HIV (15%) and those using pre-exposure prophylaxis (PrEP) (12%), compared with only 2% among individuals not taking antiviral medication. A more comprehensive and current overview will be forthcoming with the publication of data from the 2024 EMIS survey. In the interim, however, this article will rely on detailed findings from the EMIS-2017 survey [7].

Figure 1

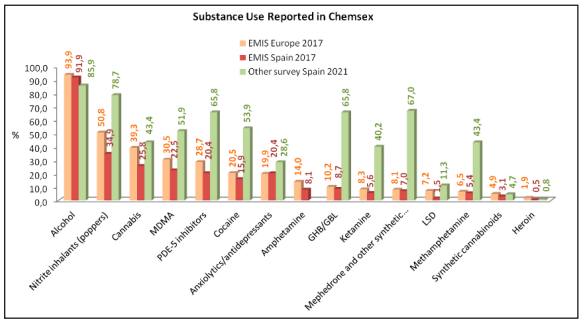


A) The percentage of nationals who used stimulant drugs to enhance sexual intensity or prolong duration ("Chemsex") in the past four weeks, based on a sample size of 50330 (source: EMIS 2024).
B) Map showing the main LGTB+ destinations in Spain. Source: FITUR LGBT+ 2024.

Spain is a leading destination for LGBT+ tourism, attracting visitors to cities like Madrid and Barcelona and smaller areas such as Ibiza (Balearic Islands), Sitges (Barcelona), Torremolinos (Málaga), and Maspalomas (Canary Islands) among others (see Figure 1B). Sexual activity serves as a significant motivator for certain travellers, with Spain ranking first (18%) in the EMIS-2010 survey of sexual behaviours abroad [8].

The global EMIS 2017 data showed that alcohol was the most commonly abused substance, with 94% of respondents reporting lifetime use. Other frequently used substances included poppers (50.8%), PDE5 inhibitors (28.7%) and anxiolytics or relaxants (19.9%). Among illicit drugs, 42% reported using them, with cannabis being the most common (39%), followed by MDMA (30.5%), cocaine (20.5%), amphetamines (14%), GHB/GBL (10.2%), ketamine (8.3%), mephedrone and other synthetic stimulants (8.1%), LSD (7.2%), methamphetamine (6.5%), synthetic cannabinoids (4.9%) and finally heroin, which had the lowest usage rate at 1.9% (see Figure 2). Additionally, 1.2% reported engaging in slamming, with crystal methamphetamine being the most frequently injected drug, followed by synthetic stimulants, ketamine and cocaine.

Figure 2



Percentage of substances reportedly ever used to enhance sexual intensity or prolong sexual duration: A comparison between data from the EMIS 2017 Reports [7,9] and the 2021 Spanish survey Aproximación al Chemsex [10].

Data from Spain's EMIS 2017 survey (N=10634) [9] reflect similar patterns. Alcohol consumption in the past year was reported by 91.9% of participants. Among recreational substances, poppers were the most frequently used (34.9%), followed by cannabis (25.8%), cocaine (15.9%), and MDMA (22.5%). Use of PDE5 inhibitors stood at 20.4%, while the prevalence of mephedrone and other synthetic cathinones (7.0%), synthetic cannabinoids (3.1%) and other stimulants (1.3%) was slightly lower. Among those who used substances other than alcohol, tobacco, or sedatives, 54.5% reported polydrug use. Within this group, 45% had used one drug, whereas 55% had used two or more.

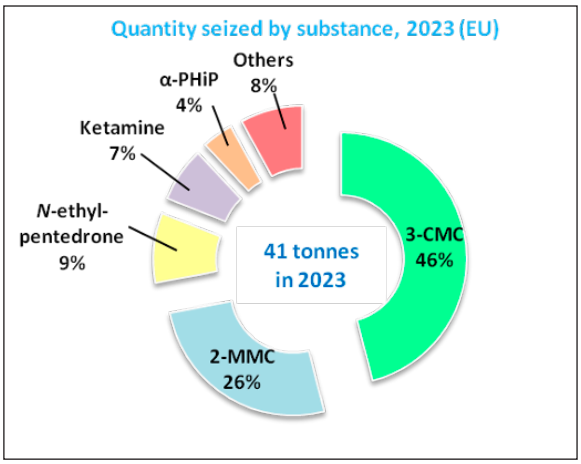
A more recent survey conducted in Spain in 2021 (N=564) [10] indicate an increase in the use of

mephedrone and other synthetic cathinones, GHB/GBL, methamphetamine and ketamine compared to EMIS 2017 data, with reported usage rates of 67.0%, 65.8%, 43.4% and 40.2%, respectively (see Figure 2). There was also a notable rise in the use of nitrite inhalants and PDE-5 inhibitors. No data from anphetamine use were reported. Additionally, a pilot study involving pre-exposure prophylaxis (PrEP) users (N = 169) found that 63% had participated in Chemsex. Common drug combinations included cocaine–PDE-5 inhibitors–ketamine, ketamine–synthetic cathinones–GHB/GBL, GHB/GBL–methamphetamine–PDE-5 inhibitors and poppers–PDE-5 inhibitors–cannabis [11].

1.1. Chemsex and New Psychoactive Substances (NPS)

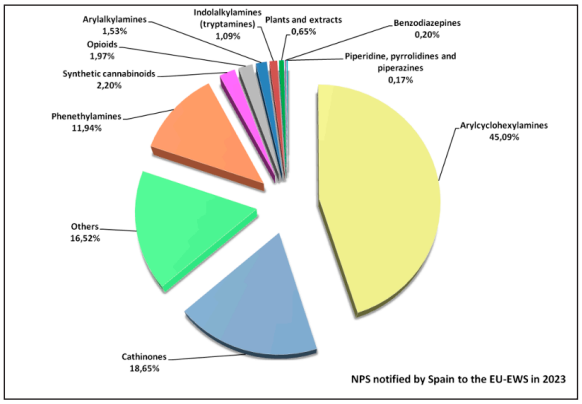
Based on findings from the EMIS surveys [6,9] and existing literature [12], the prevalence of novel psychoactive substances (NPS) in Chemsex remains relatively low, with the exception of synthetic cathinones. However, data from more recent surveys are still pending. The internet, particularly through dating apps, has had a significant impact on sexual behaviour by facilitating encounters that increase access to NPS. At the same time, data reported by the European Union Drug Agency (EUDA) [13] indicate that the number of NPS first reported in Europe has decreased in recent years, although the number of seizures and the overall quantity of NPS has not. In 2023, for instance, 41 tons of NPS were seized in Europe. As illustrated in Figure 3, the primary substances confiscated were the cathinones 3-chloromethcathinone (3-CMC) and 2-methylmethcathinone (2-MMC), which accounted 46% and 26% of the total, respectively.

Figure 3.



The total amount of NPS seized in Europe in 2023, along with the percentage distribution of substances. The 2023 Annual Situation Report (ASR) from Spain [14] included a total of 9087 cases (detections) in which one of the NPS from the EUDA notification list was identified. Of these, 6581 correspond to seizures, 996 to samples collected, and 1510 to biological samples (including 585 detections in deceased individuals). That year, the ASR from Spain included notifications of 149 different NPSs, belonging to different groups according to the EUDA classification (see Figure 4).

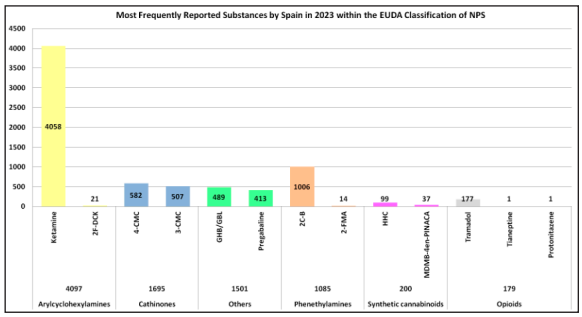
Figure 4



NPS notified by Spain to the European Early Warning System (EU-EWS) in 2023.

Regarding the substance group, arylcyclohexylamines were the most frequently notified group, due to the large predominance of ketamine, which is the most reported NPS in the ASR (4097 cases). The second most frequent group is cathinones (1695 cases). This is the most diverse group, as it includes 37 different cathinones, with the most reported being clephedrone (4-CMC) (582 cases) and 3-CMC (3-chloromethcathinone) (507 cases). The third most notified NPS group is the "Other" category (1606 cases), which includes 489 notifications of GHB/GBL and 413 of pregabalin, as the most frequent (see Figure 5).

Figure 5.



Most frequent NPSs reported by Spain in 2023 within most prevalent families (Arylcyclohexylamines, Cathinones, Others, Phenethylamines, Synthetic Cannabinoids and Opioids).

Forensic case reports highlight the notable presence of synthetic cathinones in Chemsex-related fatalities. In four cases, 4-methylethcathinone (4-MEC) was identified along with other substances such as cocaine, MDMA, ethanol, sildenafil and ethyl chloride [15-18]. Mephedrone was initially implicated in several Chemsex-related deaths [15,19]. In subsequent years, its analogue 3-MMC has been found in more recent cases [20-23]. Additionally, other substances including the cathinone 4-methyl-pentedrone [24] and

methoxphenidine [25], have been associated to Chemsex-related fatalities. GHB is also of particular concern due to its high prevalence in these deaths [16-18,26-28].

Trends in the illicit drug market will undoubtedly have a significant impact on consumption habits. With the growing influence of NPS on the drug market, these substances are expected to play an increasing role in Chemsex practices, as we await further insights from the 2024 EMIS survey. One of the main problems faced by Chemsex users is that when they consume mephedrone, methamphetamine or ketamine, they often do not know exactly what they are taking. This uncertainty arises because, due to international regulations, these substances are being replaced in the illicit market by structural analogues whose toxicity is often greater or even unknown.

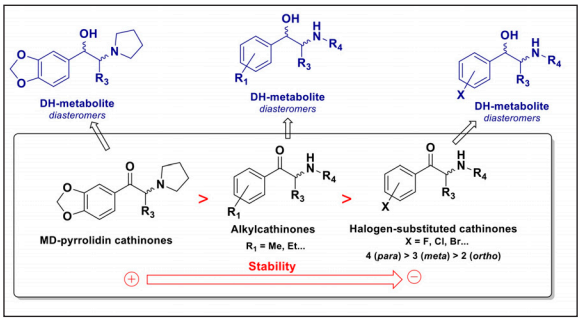
2. Analytical Challenges for Forensic and Clinical Toxicology Laboratories

The rise of NPS presents significant challenges for clinical and forensic laboratories, especially in adapting detection methods and ensuring the accurate screening of compounds and their metabolites. Key issues include limited research on substance stability, a lack of reference materials, and the necessity for continuous updates to toxicological analytical methods. NPS can induce intoxication at low doses, complicating their identification, particularly in fatal cases. The lack of data on blood levels and their role in intoxication further complicates the interpretation of analysis. Gathering information from family, friends and forensic evidence will help identify substances for testing. Key biological samples include blood, urine, gastric contents and in some postmortem cases, muscle, lung or liver tissue. Hair analysis provides insight into consumption habits and NPS exposure. Examining any paraphernalia found near the body, such as powders, liquids, tablets, syringes or vapes is essential to guide the analysis.

2.1 Samples and Storage Stability

Samples must be analysed promptly and stored at -20°C or below both before and after analysis to maintain their integrity. This requirement is especially critical given the instability of certain NPS, a concern that becomes even more evident in postmortem samples. The use of additives, such as appropriate preservatives and anticoagulants, is recommended to minimize degradation after the autopsy [29]. Special attention should be given to synthetic cathinones, since they exhibit chemical instability in biological samples such as blood and urine [30-42]. Higher pH values enhance the breakdown of cathinone analogues. Degradation pathways in alkaline solutions suggest that oxidants such as dissolved oxygen accelerate decomposition, while antioxidants may reduce this effect. The chemical stability of cathinones is affected by the substituents on their aromatic rings, as outlined by the Hammet equation [30]. Electron-donating groups such as methylenedioxy (MD) derivatives are the most stable, whereas halogen derivatives are less stable. Tertiary amines, such as pyrrolidine derivatives, are more stable than secondary aliphatic amines due to their resistance to oxidative deamination (see Figure 6).

Figure 6



Comparison of the stability of cathinones based on the nature of the substituents on the aromatic ring and on the amino group. Highlighted in blue the corresponding dihydro (DH)-metabolites.

Factors such as laboratory storage conditions, postmortem interval, ambient temperature, or cadaveric alterations may influence the detection of synthetic cathinones. This situation may lead to false negatives and inaccuracies in the reported concentrations, which might not accurately reflect their actual levels at the time of death or incident. Some researchers suggest using dihydro metabolites, formed by the reduction of the keto group, as biomarkers for cathinone exposure due to their higher stability compared to the parent drug [33-35,39,42]. Therefore, their detection in biological matrices may be a good option for assessing cathinone exposure, particularly in the case of halogenated cathinones.

2.2 Systematic Toxicological Analysis Identification of the NPS

Routine toxicological analysis begins with blood ethanol examination and screening for other volatile substances using headspace gas chromatography with flame ionization detection (HS-GC-FID). If urine samples are available, they are screened using cloned-enzyme donor immunoassay (CEDIA). Regardless of immunoassay results, all samples undergo solid-phase extraction (SPE) utilizing both reverse-phase and cation-exchange mechanisms. The extracted compounds are then analysed using gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography with diode array detection (HPLC-DAD) and liquid chromatography with high-resolution mass spectrometry (LC-HRMS) on an Orbitrap QExactive system. Once a substance is confirmed, it is quantified using liquid chromatography coupled with a triple quadrupole mass spectrometer (LC-QQQ). Urine samples are also diluted 1/5 with an aqueous mobile phase and analysed directly by LC-HRMS and LC-QQQ. When drug stashes or paraphernalia are found, priority should be given to processing

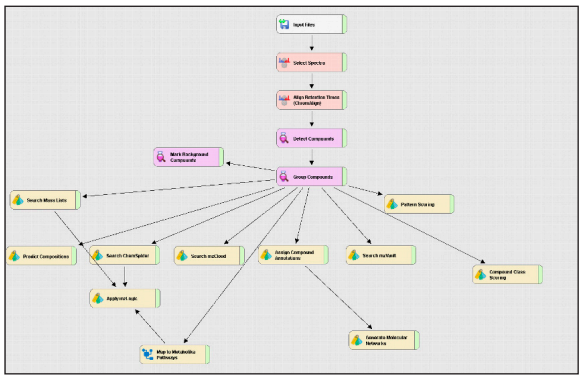
these items. Powdered or liquid substances are dissolved in organic solvents for direct analysis. Objects such as syringes, spoons, paper wrappers or smoking pipes are cleaned with organic solvents before being analysed using the aforementioned techniques.

A mandatory screening strategy for non-targeted analysis of NPS in biological matrices involves the use of LC-HRMS/MS and/or GC-MS techniques, with regularly updated spectral libraries. An analytical chromatographic and spectrometric methodology previously developed for multitarget substance screening in biological fluids was used [43,44]. The UHPLC-HRMS/MS method employed a Thermo Vanquish UHPLC system with an AccuoreTM phenyl-hexyl column maintained at 40ºC. The mobile phases included solutions with ammonium formate and formic acid, with elution performed at a gradient flow rate of 0.5 mL/min. The detection system was a Q Exactive HF mass spectrometer with electrospray ionization (HESI-II), operated in non-targeted discovery mode. The Full Scan (FS) parameters were configured as follows: a resolution of 35000 (FWHM), an automatic gain control (AGC) target of 1e6, a maximum injection time (IT) of 120 ms and a scan range of m/z 130–650. For data-dependent MS/MS (ddMS2), the settings included fragmentation of the three most intense ions detected in each FS scan (top 3 fragmentation), a resolution of 17500 (FWHM), an AGC target of 5e4, an IT of 50 ms and an isolation window of m/z 1.0.

Fragmentation was performed using ramped normalized collision energy (NCE) of 30, 60 and 90 eV. Spectral data were recorded in profile mode, with acquisition employing a dynamic exclusion period of 9.0 seconds and an exclusion list incorporating principal ions from the mobile phase with a retention time window of ±0.5 minutes.

The systematic processing for non-targeted acquisition in HRMS was accomplished using Compound Discoverer software 3.3 SP2 (ThermoScientific) through a workflow data processing. The workflow used (see Figure 7) uses detection nodes based on searches in libraries such as mzCloud, MassBank and NIST, as well as lists of exact masses of NPS in ChemSpider or downloaded from HighResNPS and SWDrug. It also incorporates the mapping of the metabolism of various NPS and the search for characteristic diagnostic ions and neutral losses [45-47]. The identification of NPS and their metabolites is further complemented by nodes that generate molecular formulas from the exact masses of precursor ions using isotopic profiles. Notably, the process is enhanced by the molecular networking node, which is particularly helpful for grouping the different MS2 spectra contained in the acquisition raw file based on their similarities. The results are displayed as a molecular network, where each cluster represents a set of compounds with similar MS2 spectra that are interconnected through metabolic reactions.

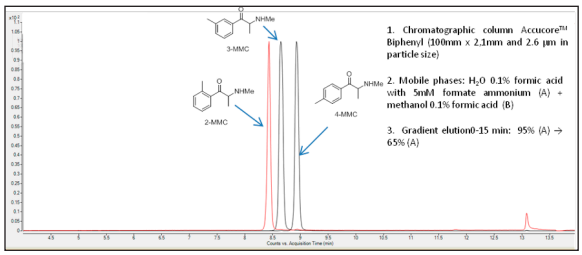
Figure 7



Workflow used for the detection of NPS and their metabolites. The different identification nodes are highlighted in yellow.

Another interesting factor to consider is testing the capability of the analytical method to separate positional isomers of NPS. This is particularly relevant for cathinones. For example, 4-methylmethcathinone (4-MMC) and 3-MMC are regulated, while 2-MMC is not. A similar situation occurs with 4-chloro-methcathinone (4-CMC) and 3-CMC compared to 2-CMC. Using a biphenyl column and an appropriate gradient enables the separation of positional isomers of 2-, 3-, and 4-MMC (see Figure 8); similar to what happens with CMC. This column type leverages **ππ-ππ** interactions and unique selectivity characteristics, making it particularly suitable for distinguishing structurally similar compounds such as positional isomers [48].

Figure 8



Resolution of positional isomers of MMC (methylmethcathinone) can be effectively achieved using a biphenyl stationary phase column. 2-Methylmethcathinone involved in some of the cases reported is highlighted in red.

Although these techniques are also valuable for analyzing paraphernalia, other powerful methods, such as proton nuclear magnetic resonance (NMR) and FT-IR (Fourier Transform Infrared) spectroscopy can serve as complementary tools. Notably, NMR is particularly effective for resolving positional isomers of cathinones, as will be discussed further in the case reports [49].

3. Results and Discussion

Table 1 summarises the Chemsex-related cases analysed in our laboratory from the Canary Islands region over the past three years (2021–2024).

Cases 1–7 are postmortem, while cases 8 and 9 involve in vivo intoxications resulting from Chemsex activities, as reported by emergency units. As previously mentioned, routine toxicological analysis included non-targeted screening using GC/MS, HPLC-DAD and UHPLC-HRMS/MS, along with targeted analysis for quantification using liquid chromatography-tandem mass spectrometry (LC-QQQ). Additional methods, such as 1H-NMR, were employed for cases 2 and 3. All cases, except for cases 7 and 9, have been reported to the EU Early Warning System (EUDA), with cases 7 and 9 still in progress, pending the receipt of reference standards for the involved NPS.

Table 1 Summary of the presented Chemsex cases.

Cases	History	Samples	Substances found in blood	Other findings
1	25-year-old man discovered deceased in an area known for cruising.	Fb, vh, ur, gc, paraphernalia	Alcohol: 1.67 g/L (1.64 g/L in vitreous humour) GHB: 412 mg/L	Mephedrone, ketamine and norketamine, GHB (737 mg/L) in urine. Mephedrone and GHB in gastric content.
2	60-year-old man died after injecting "3-MMC" during a Chemsex session.	Fb, vh, ur, bag with white powder	3-MMC: 50 ng/mL, metoprolol: 5 ng/mL, sildenafil: 6 ng/mL, nevirapine and emtricitabine.	3-MMC (vh and urine). DH-3-MMC and nor-3-MMC were found in urine along with sildenafil, metoprolol, nevirapine and emtricitabine.
3	59-year-old man found naked in bed with an anal dildo.	Pb, white powder	3-CMC: 2 ng/mL, citalopram/escitalopram: 80 ng/mL, *N*-desmethylcitalopram: 50 ng/mL.	---
4	63-year-old man died after consuming poppers and injecting an unknown substance.	Pb, vh, ur.	3-CMC: 7.4 ng/mL, amiodarone: 434 ng/mL, atropine: 96.9 ng/mL, emtricitabine.	3-CMC (vh and ur), DH-3-CMC (vh and ur), DH-nor-3-CMC (vh and ur), nor-3-CMC (vh and ur), ramipirilate (ur) and emtricitabine (ur).
5	48-year-old man found agitated by the police at a gas station near a known cruising area. He died en route to the hospital.	Fb, vh, ur, gc.	DH-3-CMC, DH-nor-3-CMC, nor-3-CMC, midazolam: 386 ng/mL, atropine: 30.8 ng/mL, naloxone, flumazenil.	3-CMC only found in gc.
6	44-year-old man with a history of polydrug abuse was found dead in a recreational sexual setting. Consumption of popper prior to his death.	Fb, vh, ur, gc and bottle.	Isopropyl alcohol: traces, amphetamine: 7 ng/mL, methamphetamine: 4 ng/mL, sildenafil: 238 ng/mL, 11-carboxy-THC: 66.7 ng/ mL.	Cobicistat and darunavir in urine. Isopropyl nitrite in bottle.
7	45-year-old man, found deceased in a hotel while engaged in sexual activity and using stimulant drugs.	Fb, ur and paraphernalia	GHB: 22.13 mg/L, 2-fluoro-deschloroketamine (2F-DCK), DCK, 2-MMC: 57.38 ng/mL, methamphetamine: 41.87 ng/mL, amphetamine: 6.97 ng/mL, ketamine: 16.18 ng/mL, norketamine: 4.94 ng/mL, phentolamine, papaverine and sildenafil: 4.20 ng/mL	Urine findings: GHB (36.85 mg/L), 2-MMC, DH-2-MMC and nor-2-MMC, sildenafil, 2F-DCK, DCK, ketamine and metabolites, methamphetamine and amphetamine, phentolamine and papaverine.
8*	44-year-old man found in coma. His companion reported the consumption of GHB the day before for sexual activity. He was traNPSorted to the hospital, where he made a full recovery	Serum, b, ur	GHB: 326.51 mg/L in serum, blood: diazepam: 3.8 ng/mL, nordiazepam: 9.2 ng/mL, oxazepam: 3.2 ng/mL, temazepam: 1.3 ng/mL, midazolam, sildenafil: 0.5 ng/L, naloxone, abacavir, dolutegravir.	Urine findings: GHB (1713.92 mg/L), 3-CMC, DH-3-CMC, nor-CMC, sildenafil, diazepam, nordiazepam, oxazepam, temazepam, naloxone and etomidate, abacavir, dolutegravir.
9*	Man found unconscious in a darkroom. He was traNPSorted to the hospital, where he made a full recovery	Serum, b, ur	*N*-ethyldeschloroketamine (2-oxo-PCE), emtricitabine, rilpivirine, flumazenil, laudanosin, midazolam.	Urine findings: 2-MMC, DH-2-MMC and nor-2-MMC, 2-oxo-PCE and metabolites. Emtricitabine, flumazenil, naloxone, laudanosine, *N*-dealkylflurazepam, midazolam and α-hydroxy-midazolam were present

*Fb: femoral blood, b: blood, Pb: peripheral blood, vh: vitreous humour, ur: urine, gc: gastric content. *in vivo cases.*

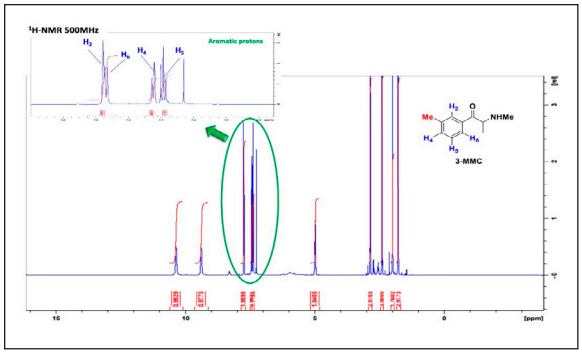
Case 1: A 25-year-old man was found dead in a cruising area, with a bottle of whiskey, a plastic container with a clear liquid and several empty bottles nearby. The autopsy revealed foam in the mouth, acute pulmonary oedema and brain haemorrhaging. Blood, urine, vitreous humour, stomach contents and paraphernalia were collected for toxicological analysis. The clear liquid in the bottle was confirmed to be GHB, which directed further testing. Toxicological results showed a blood alcohol concentration of 1.67 g/L and a GHB level of 412 mg/L. Urine analysis revealed the presence of GHB, mephedrone, ketamine, and norketamine, while both GHB and mephedrone were found in the stomach. The elevated GHB levels suggested fatal intoxication. The cause of death was determined to be respiratory failure, likely from the combined depressive effects of alcohol, GHB and ketamine.

Case 2: A 60-year-old man died during a Chemsex session after injecting a substance identified as 3-MMC. Despite effort to perform CPR techniques, could not be revived. GC-MS and LC-HRMS/MS identified the white powder as methylmethcathinone, which was further confirmed as 3-MMC through 1H-NMR (500 MHz) (see Figure 9) [50].

The 1H-NMR of 2-, 3 and 4-substituted aromatic compounds have characteristic patterns, which are used to distinguish 2-, 3- and 4-MMC. 3-MMC has H-2 appearing as a singlet. Both H-6 and H-4 appear as doublets of doublets (ortho split) and H-5 appears as a triplet (di-ortho split). The only structure that has an isolated aromatic proton that does not couple with adjacent protons is the substitution at carbon 3, corresponding to 3-MMC.

Toxicological findings revealed the presence of 3-MMC at 50 ng/mL, along with metoprolol, sildenafil and antiretroviral drugs in his blood. Although the detected concentration of 3-MMC is lower than that reported in other cases [20,21,51], it is important to consider the deceased's age and the presence of cardiovascular issues, as evidenced by the concomitant presence of metoprolol. The cause of death was determined to be an acute myocardial infarction due to the toxicity of 3-MMC.

Figure 9

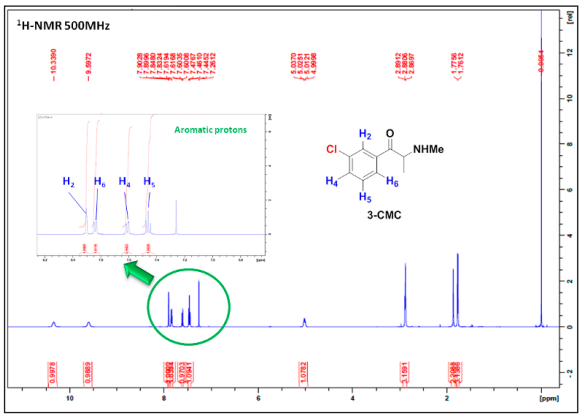


[1]H-NMR spectra of the white powder found near the corpse of case 2 prepared in CDCl3.

Case 3: A 59-year-old man was found deceased in his bed with an anal dildo, and a white powder was discovered on the bedside table. The following day, an autopsy was conducted, and peripheral blood samples, along with the white powder, were sent for toxicological analysis. The powder was identified as chloromethcathinone (CMC) using GC-MS and LC-HRMS/MS, with 1H-NMR (500 MHz) confirming it as 3-CMC (see Figure 10). In a similar way to what was previously described for 3-MMC, 3-CMC has H-2 appearing as a singlet. Both H-6 and H-4 appear as doublets of doublets (ortho split) and again H-5 appears as a triplet (di-ortho split). The only structure

that has an isolated aromatic proton that does not couple with adjacent protons is the substitution at carbon 3, corresponding to 3-CMC.

Figure 10



^{1}H-NMR spectra of the white powder found near the corpse of case 3 prepared in CDCl3.

Toxicological analysis revealed 3-CMC (2 ng/mL), its metabolite dihydro-3-CMC and antidepressants citalopram/escitalopram (80 ng/mL) and N-desmethylcitalopram (50 ng/mL). The low levels of 3-CMC detected were of concern due to the known instability of halogenated cathinones in biological samples. Compared to previous postmortem cases, 3-CMC blood concentrations typically ranged from 10 to 2800 ng/mL [51], with dihydro-3-CMC serving as a stable intake biomarker. The cause of death was probably a vasovagal reaction caused by the ingestion of toxic substances.

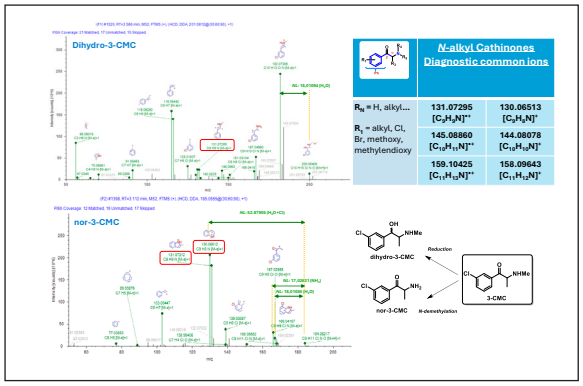
Case 4: A 63-year-old man died after using poppers and injecting a substance, as reported by his partner. Two syringes and a metal pipe were discovered in the trash. The deceased had a history of HIV. An autopsy conducted the next day revealed anatomical findings of atheromatous plaques in the circumflex coronary artery (50% occlusion) and almost complete occlusion of the right coronary artery. Samples of peripheral blood, vitreous humour and urine were collected for toxicological analysis. A liquid chromatographic method using a biphenyl column was employed to separate various cathinone regioisomers as previously described. The toxicological results indicated the presence of 3-CMC, amiodarone and atropine (the latter two as a result of resuscitation attempts) at concentrations of 7.4, 434 and 96.9 ng/mL respectively, along with the retroviral drug emtricitabine. These substances were also detected in the vitreous humour and urine. Additionally, metabolites of 3-CMC, such as dihydro-3-CMC, dihydro-nor-3-CMC and nor-3-CMC, were found. The cause of death was determined to be acute heart failure.

Case 5: A 48-year-old man was found by the police at a gas station near a cruising area. Upon ambulance´s arrival, he lost consciousness and was transported to the hospital. During the transfer, he experienced severe bradycardia that did not respond to medication. Despite attempts at cardiac massage and defibrillation, he went into asystole and passed away. An autopsy was performed the following day and the toxicological results revealed the presence of dihydro-3-CMC, nor-3-CMC (see Figure 11) and dihydro-nor-3-CMC in blood, vitreous humour and urine while 3-CMC was only detected in the gastric content.

The presence of midazolam, atropine, naloxone and flumazenil was attributed to the medical treatment administered prior to death.

There is limited information on the acute toxicity of 3-CMC. However, based on the available data, the adverse effects are likely to share similarities with other closely related synthetic cathinones. In cases of poisoning, these similarities include the sympathomimetic toxidrome. This case emphasises the importance of identifying cathinone metabolites by focusing on diagnostic ions and characteristic neutral losses during data mining using specialised data processing software.

Figure 11



MS^2 spectra of 3-CMC metabolites, dihydro-3-CMC (top) and nor-3-CMC (bottom) from case report 5. Some of the diagnostic ions and neutral losses are highlighted. For further information [45,47].

Case 6: A 44-year-old man with a history of polydrug abuse was found deceased in a recreational sexual setting after using poppers. GC-MS analysis of a nearby liquid identified isopropyl nitrite and acetone (a degradation product). Toxicological analysis of blood revealed traces of isopropyl alcohol, likely from popper exposure, as well as amphetamine, methamphetamine, sildenafil and 11-carboxy-THC, with concentrations of 7, 4, 238 and 56.7 ng/mL, respectively. These substances were also detected in the vitreous humour and urine. Cobicistat, a CYP3A4 isoenzyme inhibitor that boosts certain antiretroviral medications like darunavir, was also present. Alkyl nitrites can interact with PDE5 inhibitors, leading to dangerously low blood pressure, while amphetamine derivatives raise it, this can lead to cardiovascular collapse, arrhythmias and heart failure. This risk is heightened by cobicistat's inhibition of CYP3A4, which is responsible for metabolizing sildenafil.

Case 7: A 45-year-old man was found deceased in a hotel while engaged in sexual activity and using stimulant drugs. Blood, urine and paraphernalia were collected for toxicological analysis (see photograph 1). GC-MS and LC-HRMS/MS analysis of the paraphernalia showed the presence of GBL in the droppers, 2-MMC in one vial with a diffuser valve and a mixture of 2-fluoro-deschloroketamine (2F-DCK), deschloroketamine (DCK) and ketamine in another. The topaz bottles labelled FIST were found to contain amyl nitrite, while those from the Highrise brand contained pentyl nitrite. The last vial contained a commercial preparation of papaverine and phentolamine, known as Skat therapy, for the treatment of erectile dysfunction. The pale blue powder was identified as a mixture of 2F-DCK, DCK and ketamine, whereas the white powder consisted solely of 2-MMC.

Photograph 1

Paraphernalia found near the corpse in case 7. Two vials with powdery substances, three topaz vials containing what appear to be inhalant nitrites (poppers), one vial for erectile dysfunction (SKAT therapy containing papaverine and phentolamine), two vials containing a liquid substance with droppers and two other vials with a diffuser for anal introduction.

Biological samples, including blood and urine, were submitted for routine toxicological analysis. GHB was detected in blood and urine at concentrations of 22.13 and 36.85 mg/L, respectively, while 2-MMC, methamphetamine, amphetamine, ketamine and norketamine were quantified at levels of 57.38, 41.87, 6.97, 16.18 and 4.94 ng/mL respectively. Quantification of 2F-DCK and DCK is still pending, as the corresponding reference standards are not yet available.

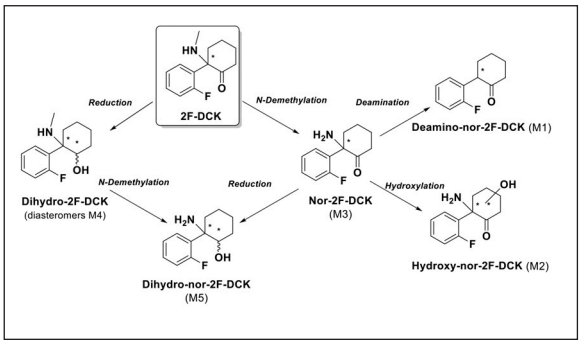
In-depth HRMS data mining was conducted, leveraging previously described metabolomic pathways for 2F-DCK [50]. Additionally, diagnostic fragment ions for the arylcyclohexylamine core proved useful for detecting this class of compounds [45]. Several metabolites were tentatively identified in urine, as displayed in Table 2, and these findings align with previously reported in vivo and in vitro results [52]. The detected metabolites can be explained according to the proposed metabolic pathway illustrated in Scheme 1.

Table 2

Name	Formula	RT (min)	*m/z* observed	Δppm	Peak areas (counts)	Main observed fragments
2F-DCK	$C_{13}H_{16}FNO$	3.58	222.12827	-2.70	6.24E+9	221.12796; 204.11749; 191.08603; 163.09108; 147.05994; 135.05997; 125.03950; 115.05396; 109.04474
Deamino-nor-2F-DCK (**M1**)	$C_{13}H_{15}FO$	3.54	193.10196	-1.86	1.38E+8	193.10300; 175.09190; 147.05980; 125.03943; 109.04475; 97.04482
Hydroxy-nor-2F-DCK (**M2**)	$C_{12}H_{14}FNO_2$	4.57	224.10764	-2.18	1.55E+7	224.10754; 191.08646; 178.10222; 163.09193; 135.06021; 109.04488
Nor-2F-DCK (**M3**)	$C_{12}H_{14}FNO$	3.44	208.11261	-2.93	5.03E+8	208.11244; 191.08612; 163.09116; 163.09116; 153.06927; 135.05994; 125.03950; 109.04476
Dihydro-2F-DCK (diasteromer 1) (**M4**)	$C_{13}H_{18}FNO$	3.42	224.14417	-1.56	4.25E+8	224.14330; 193.10190; 175.09128; 149.07559; 147.06053; 125.03966; 109.0488; 97.04498
Dihydro-2F-DCK (diasteromer 2) (**M4**)		3.99			3.73E+8	
Dihydro-nor-2F-DCK (**M5**)	$C_{12}H_{16}FNO$	3.52	210.12837	-2.38	5.84E+8	210.12833; 193.10187; 175.09134; 149.07562; 147.06010; 125.03957; 109.04486; 97.04495

2F-DCK and main metabolites tentatively identified in urine after hydrolysis and SPE.

Scheme 1



The proposed metabolic pathway for 2F-DCK to explain the metabolites found in the samples analyzed in case 7.

2F-DCK is more lipophilic than ketamine due to the substitution of chlorine by fluorine, but its pharmacological and toxicological profiles remain largely unknown. Users report effects similar to ketamine, including dissociation, anaesthesia and analgesia without loss of consciousness. A poisoning outbreak in Hong Kong linked to 20 confirmed cases of 2F-DCK intoxication revealed neurological (agitation, delirium, convulsions) and cardiovascular symptoms (hypertension, tachycardia). The effects are comparable to ketamine, with excessive cardiovascular stimulation and fatalities mainly attributed to respiratory depression [52,53]. Pending quantification of 2F-DCK and DCK levels, it appears that the combination of ketamine, 2F-DCK, DCK, GHB, methamphetamine and 2-MMC may have caused an acute cardiac episode leading to the death of the individual.

Case 8: A patient was hospitalized due to severely impaired consciousness, with his partner stating that he had consumed GHB the previous day during sexual activity. Toxicological analysis of blood

revealed a GHB concentration of 326.51 mg/L, along with diazepam, nordiazepam, oxazepam, temazepam and sildenafil at various levels. Midazolam and naloxone were also detected. In urine, GHB was found at a much higher concentration (1713.92 mg/L), along with 3-CMC, dihydro-3-CMC, nor-3-CMC and the rest of substances.

Case 9: A live patient was found in a dark nightclub room with a diminished level of consciousness. The patient was hospitalised on suspicion of GHB and popper use in a Chemsex context. Treatment included flumazenil, naloxone, midazolam, cisatracurium and propofol. The time elapsed between the incident and the sample collection is unknown. Initial clinical drug tests yielded negative results. Blood, serum and urine samples were collected for toxicological analysis. Analysis of blood and serum detected N-ethyl-deschloroketamine (2-oxo-PCE) and its metabolites, antiretroviral drugs emtricitabine and rilpivirine, as well as flumazenil, laudanosine and midazolam. Urine analysis revealed the presence of 2-MMC, dihydro-2-MMC, nor-2-MMC, 2-oxo-PCE and its metabolites and also emtricitabine, flumazenil, naloxone, laudanosine, N-dealkylflurazepam, midazolam and α-hydroxymidazolam. Notably, no traces of GHB were detected in any of the samples.

2-oxo-PCE is an arylcyclohexylamine derivative that shares the core structure of 2-phenyl-2-aminocyclohexanone with ketamine. However, 2-oxo-PCE differs from ketamine by the absence of a chlorine atom and the presence of an ethyl group on the amine. The primary clinical symptoms associated with 2-oxo-PCE include impaired consciousness, confusion, abnormal behaviour, hypertension, tachycardia and convulsions. Its toxicity seems to be more severe than ketamine, as it appears to be more potent at lower doses. The concurrent use of 2-oxo-PCE with other substances, such as ketamine, cathinones or antidepressants, may increase the risk of toxicity [54-58].

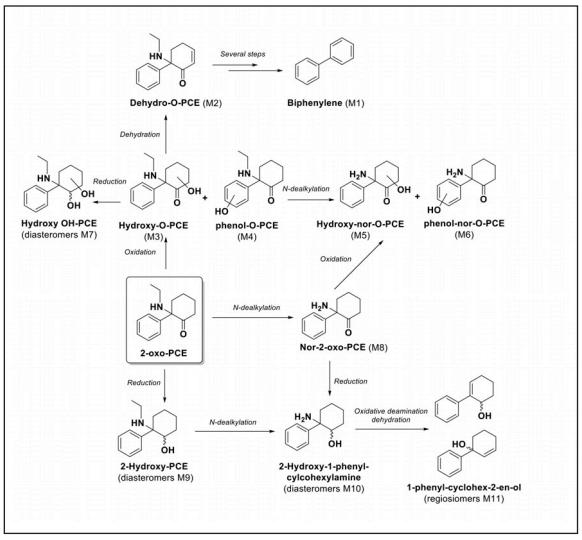
Similar to case 7, HRMS data were processed using Compound Discoverer software, utilizing previously described metabolic pathways for 2-oxo-PCE [57]. Again, diagnostic fragment ions for the arylcyclohexylamine core were useful in detecting this class of compounds [45]. Several metabolites were tentatively identified in urine, as shown in Table 3, and these findings are consistent with previously reported in vivo and in vitro results [57]. The detected metabolites can be explained according to the proposed metabolic pathway illustrated in Scheme 2. Hydroxy-O-PCE (M3) and phenol-O-PCE (M4) were not detected.

Table 3

Name	Formula	RT (min)	*m/z* observed	Δppm	Peak areas (counts)	Main observed fragments
2-oxo-PCE	$C_{14}H_{19}NO$	3.53	218.15410	0.91	1.43E+11	176.09634; 145.10127; 129.06998; 117.07025; 91.05465; 67.05490
Biphenylene (**M1**)	$C_{12}H_{10}$	2.65	155.08562	0.58	7.75E+7	155.08557; 153.06999; 129.07005; 128.06229; 115.05457
Dehydro-O-PCE (**M2**)	$C_{14}H_{17}NO$	3.39	216.13846	0.78	5.43E+8	198.12819; 171.08047; 152.06209; 143.08565; 141.06990; 128.06229; 115.05452; 91.05460
Hydroxy-nor-PCE (**M5**)	$C_{12}H_{15}NO_2$	4.15	206.11772	0.77	2.27E+8	188.10722; 173.09607; 160.11218; 145.10094; 129.07004; 117.07012; 115.05441; 91.05467; 67.05490
Phenol-nor-PCE (**M6**)	$C_{12}H_{15}NO_2$	3.14	206.11771	0.73	3.37E+8	189.09122; 161.09633; 145.06442; 107.04951; 91.05470; 79.05479; 67.05486
Hydroxy-OH-PCE (**M7**)	$C_{14}H_{21}NO_3$	2.98	236.16452	0.04	5.23E+7	173.09618; 145.10149; 117.07024; 115.05444; 105.03391; 91.05471; 67.05485
Nor-2-oxo-PCE (**M8**)	$C_{12}H_{15}NO$	3.26	190.12270	0.31	1.83E+10	173.09616; 155.08578; 145.10123; 129.07002; 117.07014; 91.05469; 67.05483
2-Hydroxy-PCE (diasteromer 1) (**M9**)	$C_{14}H_{21}NO$	4.02	220.16975	0.72	8.54E+9	175.11850; 157.10123; 129.07001; 91.05470; 79.05477; 69.07046
2-Hydroxy-PCE (diasteromer 2) (**M9**)		4.25	220.16972	0.59	1.08E+10	175.11203; 157.10094; 129.07036; 91.05470; 79.05479; 69.07046
1-Phenyl-cyclohex-2-en-ol (3 regiosiomers) (**M11**)	$C_{12}H_{14}O$	4.26	175.11192	1.03	4.06E+9	157.10156; 142.07813; 129.06999; 107.04952; 91.05471; 79.05479; 69.07054
		4.03	175.11192	1.03	6.98E+9	157.10430; 142.07794; 131.08591; 129.07005; 107.04951; 91.05473; 79.05479; 69.07051
		3.20	175.11182	0.46	4.47E+9	157.10161; 142.07761; 129.07036; 115.05455; 107.04946; 91.05466; 79.05478; 69.07044

2-oxo-PCE and main metabolites tentatively identified in urine.

Scheme 2



Proposed metabolic pathway to explain the observed metabolites and the MS2 spectra cluster identified in the molecular network corresponding to 2-oxo-PCE metabolites.

When comparing the urinary metabolites detected in our case report with those in previous studies [57], it is important to consider the time elapsed between the events, hospital admission and sampling, as well as the detoxification treatments the patient underwent during admission.

4. Limitations

Before discussing the conclusions, it is important to highlight some limitations. The comparison involves 9 cases, 7 of which are postmortem.

While this represents a small sample size that makes drawing definitive conclusions difficult, these cases may still provide valuable insights into the evolution of the NPS market in Chemsex practices.

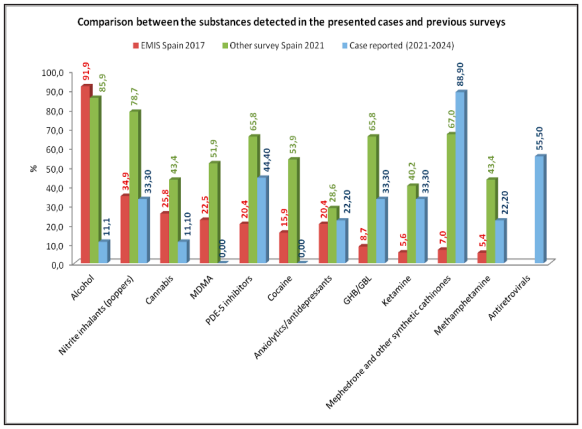
Additionally, the findings presented in this manuscript are based on toxicological analyses, in contrast to the self-reported data from previous surveys involving living individuals. However, certain substances—such as alkyl nitrites—are difficult to detect in postmortem cases due to their rapid hydrolysis in blood into inorganic nitrite, which is responsible for their toxic effects. This inorganic nitrite is further broken down after reacting with haemoglobin in erythrocytes, potentially leading to an underrepresentation of these substances in the cases analysed.

Unfortunately, data from the EMIS 2024 survey is not yet available [7]. As a result, comparisons must rely on the 2017 survey findings [6,9] and other more recent studies [10], which may no longer accurately reflect current patterns of drug and NPS use in Chemsex contexts due to the time elapsed.

5. Conclusions

The toxicological findings from the reported case studies are presented in Figure 12. Cathinones were identified in 8 out of 9 cases (88.8%), representing an increase compared to previous surveys on Chemsex. Although antiretroviral drugs were not included in the previous surveys, they emerged as the second most frequently detected substance in our casuistry (55.5%), underscoring their toxicological relevance due to potential drug interactions. The prevalence of PDE5 inhibitors, GHB/GBL and amphetamine use was higher than in EMIS 2017 [9] but lower than in the 2021 Spanish Survey [10]. Surprisingly, MDMA and cocaine were not detected in any of our cases and alcohol and cannabis were each detected in one case (11.1%). Additionally, ketamine and its analogues were detected in 3 out of the 9 cases (33.3%), highlighting their potential as emerging drugs within the Chemsex context and aligning with the 2021 Spanish Survey [10]. Similar to previous data, the percentages of anxiolytics and antidepressants were similar.

Figure 12



Toxicological results from the nine reported cases, grouped by substance type and compared to previous surveys on Chemsex [9,10].

These data show that, although the Canary Islands are considered a peripheral region of Europe, the toxicological findings of the reported Chemsex cases mirror the pattern of NPS seizures across Europe in 2023 [13], particularly as reflected in the data of the Spanish 2023 ASR [14], as well as in more recent surveys and studies conducted in the country [10-12]. In addition, other reported postmortem cases of Chemsex [15-28] indicate that cathinones were the most commonly detected substances. Another important observation is the increase in ketamine derivatives, which is driving the development of new structural analogues.

The forthcoming results of the EMIS 2024 survey will be essential to assess whether these postmortem findings are consistent with self-reported substance use patterns among Chemsex users.

In the context of Chemsex sessions, the substances used vary depending on their availability and legal status, a phenomenon similar to the general market of NPS. For instance, between 2007 and 2009, mephedrone (4-MMC) became popular in Europe due to its MDMA-like effects and its availability online as a "legal high." After a rise in its use, it was classified as a controlled substance and banned in Spain in 2011 [59]. However, mephedrone remains common in Chemsex contexts, although not everything sold as Mephedrone is 4-MMC. Following its regulation, 3-MMC, another positional isomer, began to gain traction. This substance was closely monitored by the EUDA and after international regulation, was banned in Spain in 2023 [60]. Consequently, in the observed Chemsex cases in 2024, the substance labelled as mephedrone is not 4-MMC but 2-MMC, the only remaining regioisomer that hasn't been regulated yet.

A similar trend is observed with chlorinated cathinones. 4-CMC was banned in 2021 [61], followed by 3-CMC in 2023 [62]. It is expected that 2-CMC, which is still with unregulated will begin to emerge in the market.

Ketamine and GHB are banned in Spain [63,64], but this does not prevent ketamine, diverted from its anaesthetic use in surgery or veterinary applications, from ending up on the illicit market. Recently, several synthetic arylcyclohexylamines, such as 2F-DCK, DCK, and 2-oxo-PCE, have been sold as ketamine, posing a significant risk as users may unknowingly consume potentially lethal mixtures. Similarly, GHB is often consumed in the form of GBL, which, once ingested, is converted to GHB through serum lactonase activity.

As noted in reported cases, Chemsex sessions often involve the use of multiple substances, many of which

carry significant risks. For example, the combination of alkyl nitrites and PDE-5 inhibitors can lead to severe hypotension. Simultaneously, using multiple stimulants such as amphetamine derivatives and cathinones increases the likelihood of cardiac complications. Likewise, combining central nervous system depressants such as alcohol, GHB/GBL and ketamine can result in dangerous outcomes. It is also important to highlight that many individuals participating in Chemsex are undergoing antiretroviral treatment. These medications often inhibit hepatic microsomal enzymes, altering drug metabolism and affecting their half-lives, further compounding the risks associated with substance use during these sessions [65].

Certain NPS are known to be unstable in biological matrices and can cause fatalities at low doses. Therefore, it is advised to keep samples frozen at -20ºC until analysis to prevent in vitro degradation. In the case of cathinones, it is strongly recommended to include dihydro metabolites in routine analyses as they exhibit greater stability than the parent drug.

From an analytical standpoint, Chemsex cases are likely to involve NPS, making it essential for analytical methods to stay current with libraries capable of detecting substances circulating in the market. When using HRMS to collect data, it is advisable to employ data-dependent acquisition (DDA) or data-independent acquisition (DIA), as these strategies enable the use of characteristic diagnostic fragment ion strategies, which are helpful for identifying structural cores linked to specific NPS types. Additionally, molecular networks serve as an effective tool for detecting metabolites. Combined with neutral loss strategies, these approaches - highlighted in the case reports - can aid in reconstructing the parent molecule. The analytical strategy should not only focus on detecting new NPS in the market but also on identifying their metabolites.

In terms of prevention and treatment policies, the problematic use of substances in Chemsex impacts physical, mental and sexual health, requiring a comprehensive, multidisciplinary approach that combines psychiatric, psychological, medical, sexual and therapeutic leisure care. Warning signs such as agitation, psychosis, respiratory depression, recurrent sexually transmitted infections (STIs), new diagnoses of HIV or hepatitis C or substance use disclosed during consultations may indicate the need for intervention.

Public intervention strategies should utilise a holistic, interdisciplinary approach involving professionals from mental health, medicine, sexology and addiction services, while also focusing on strengthening personal skills such as self-esteem and emotional regulation [66].

Vulnerability to Chemsex is heightened by factors such as HIV diagnosis, migration, trauma, loneliness and untreated comorbidities. Identifying these risk factors allows for more tailored and effective interventions. Peer support, therapeutic groups, healthy leisure activities and engagement with close networks are key components of psychosocial support [67,68].

In some cases, a psychopharmacological approach may be required to address mental health issues such as anxiety or depression, with careful monitoring of medication especially in HIV-positive individuals [65]. Sexual health interventions should involve personalised education and focus on dysfunctions related to drug use.

Support resources differ depending on the context and can include Addiction Treatment Centres (ATCs), Non-Governmental Organisations (NGOs) and specialised services in fields such as medicine, psychology, sexology and social work. In Spain, the NGO Energy Control provides harm reduction services such as substance testing and psychosocial support at events [69]. Healthcare resources also consist of community-based organisations and public services offering multidisciplinary treatments, although challenges remain in professional training and access to reliable information.

Specific initiatives have been implemented, including the distribution of informational, educational, and communication (IEC) materials, as well as preventive items, targeting community organisations, saunas, sex clubs and other businesses serving the gay community. Awareness is raised through websites, social media, dating apps and public spaces like streets, parks, saunas and other nightlife venues, specifically targeting GBHSH (gay, bisexual, and other men who have sex with men). Preventive information about Chemsex is also disseminated through rapid HIV and STI testing services in primary care centres, mobile units and STI centres. STI centres address the sexual health needs related to Chemsex, while some ATCs have been designated as reference centres for Chemsex care.

Public healthcare services also address the health and socio-health needs of Chemsex users. Specific training on Chemsex is provided to professionals working in ATCs, Comprehensive Addiction Centres (CAID), emergency departments, LGBT+ community organisations, addiction specialists, public health professionals and sexual health teams. Additionally, Chemsex-related questions are included in screening tools for rapid HIV and STI testing programmes and in ATCs.

Finally, data from postmortem cases, customs seizures, clinical toxicology, law enforcement and the European Union Drugs Agency (EUDA) help to understand the substances circulating in the market, assisting in the establishment of controls and raising alerts about consumption patterns and associated risks, as outlined in the annual reports produced by

the National Plan on Drugs (PNSD). The National Plan on Drugs (PNSD) is a government initiative aimed at coordinating and strengthening the policies carried out by various public administrations and social entities in Spain concerning drugs, in line with the current state of drug addiction [70].

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CAREER SPOTLIGHT:

A. Prof. Ghadeer Abdelaal M.B.B.Ch., MSc, MD, Fellow of EFMA

Associate Professor of Forensic Medicine and Toxicology, Faculty of Medicine, Zagazig University, Egypt

Current Practice Areas/Industries:
I am currently an Associate Professor of Forensic Medicine and Toxicology at Zagazig University. I am also a Fellow of the Egyptian Forensic Medicine Authority (EFMA) in Cairo. Additionally, I serve as an Associate Editor for the Egyptian Journal of Forensic Sciences (EJFS), which is the leading and only international journal dedicated to forensic and toxicology research in the Middle East and Africa.
I also hold a part-time Associate Professor position at Badr University in Cairo (BUC) since October 2023. Additionally, I am a Senior Consultant at the Forensic Medical Consultation Center at Zagazig University and a founding member of the Zagazig Forensic and Clinical Toxicology Research Laboratory (ZFCTRL) and the Safe Woman Clinic at Zagazig University Hospitals.

My practice encompasses leading-edge research and practical application across forensic medicine and toxicology. I leverage expertise in complex areas like postmortem toxicology, forensic autopsy, detailed forensic pathology, clinical forensic examinations and medico-legal report writing.
My roles extend to advanced forensic disciplines, including DNA fingerprinting and molecular forensics. I am also a dedicated academic, teaching medical students, and supervising postgraduate theses. Furthermore, I served as Strategic Planning and Resources Coordinator at the Faculty's Quality Assurance Unit and as the Postgraduate Quality Coordinator of Forensic Medicine and Clinical Toxicology and participated in the elaboration of the Zagazig university's strategic plan and in the Continuous Improvement and Qualifying for Accreditation Project (ClQAP).

Education:
My journey began with an M.B.B.Ch. in Medicine and Surgery from Zagazig University, graduating with 'Excellent with Honor'. This was followed by Master's (2014) and Doctorate (2019) degrees in Forensic Medicine and Toxicology from the same institution.
A pivotal part of my development was undertaking an intensive two-year Fellowship training program after my Doctorate at the EFMA in Cairo (2020-2022). This fellowship enriched me with comprehensive hands-on experience in all facets of forensic practice and toxicology. Currently, I am undertaking the MFFLM examination, the Membership of Faculty of Forensic and Legal Medicine of the Royal College of Physicians in London.

Did you always hope to work in toxicology? What did you do in school to prepare yourself for those opportunities:
My path to toxicology was profoundly influenced during my undergraduate studies. Specifically, in my fourth year of medical school, upon taking the course in Forensic Medicine and Toxicology, I found the subject matter utterly fascinating. This early exposure sparked a profound interest that subsequently evolved from a comprehensive medical background into a focused passion for the field.

My preparation began with pursuing consecutive Master's and doctoral degrees in Forensic Medicine and Toxicology, where both my theses extensively explored critical toxicology topics, sparking my interest in forensic toxicology, postmortem toxicology, and advanced analytical techniques like GCMS.

What work did you do following your initial undergraduate and postgraduate training if applicable? What further trainings did you undertake:
Following my graduation, I advanced through academic ranks at Zagazig University from Demonstrator to Associate Professor, during which I was involved in laboratory work, contributing to the establishment of the ZFCTRL and the development of its Standard Operating Procedures (SOP).

Beyond formal academia, I gained invaluable practical experience in toxicology through advanced training programs and workshops on drug analysis, extraction, GCMS and HRMS. To remain at the forefront of global toxicology I actively engage with the international scientific community.

This commitment is demonstrated through memberships and contributions to esteemed associations such as the International Association of Forensic Toxicologists (TIAFT), the Arab Union of Forensic and Toxicology (AUFT), the Global Academy of Forensic and Investigative Medicine and Science (GLAFIMS), and the Egyptian Society of Clinical Toxicology (ESCT). My involvement also includes active participation as an editorial board member and peer reviewer for numerous international journals, alongside collaborating with international PhD students in their research and presenting at international scientific conferences, all pivotal in fostering continuous learning and advancing the field.

What do/did you most enjoy about your work:
I am most gratified by the profound intellectual challenges and direct societal impact of my work in forensic medicine and toxicology. My enjoyment stems from advancing the field through impactful research and deciphering complex cases. Mentoring future forensic and toxicology professionals, driving initiatives like the Safe Woman Clinic to support vulnerable populations, including victims of abuse, children and those facing socio-economic disadvantage, and actively participating in international conferences and peer review to elevate global standards of justice and 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:
Reflecting on my career, I would have pursued more diverse international collaborations and specialized training opportunities earlier.

What do you like to do in your free time:
Beyond the rigorous demands of my profession, I strategically cultivate a rich and vibrant personal life; nurturing deep connections with loved ones while finding profound fulfillment in contributing to charitable causes, exploring new cultures through travel, expressing myself through music and poetry, embracing the challenge of acquiring new skills and building physical resilience through weightlifting, all of which are vital pillars supporting a truly holistic and purposeful existence.

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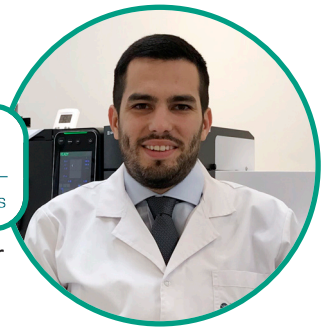

First analytical identification of N, N-dimethylpentylone in Argentina Analysis of a Seized Sample

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Microanalysis and Physical Methods in Organic Chemistry Unit (UMYMFOR) at the National University of Buenos Aires, Argentina

Keywords: New Psychoactive Substances

Abstract

The analysis of seized drugs sometimes requires different analytical techniques to allow the correct identification of the substances present. Normally, the pills circulating in Argentina contain ecstasy (MDMA) or 3,4-methylenedioxyamphetamine (MDA) and the identification of synthetic cathinones is unusual.

The appearance of new psychoactive substances requires that laboratories have the appropriate technology for their correct identification due to the large number of structural isomers that some substances present and the limited availability of reference standards.

The analysis of a seized sample using different analytical techniques is presented for the correct identification of methamphetamine and N,N-Dimethylpentylone, a synthetic cathinone identified for the first time in Argentina by the Toxicology and Forensic Chemistry Laboratory. The analysis exposes the advantage of having different analytical techniques for the identification of a synthetic cathinone that presents structural isomers and the different limitations that gas chromatography with mass spectrometry detection and electron impact ionisation (GC-IE-MS) represent for the identification of this group of substances.

Introduction

The 'new psychoactive substances' (NPS), commonly referred to as 'designer drugs' (a misnomer given the existence of natural NPS), are defined by the United Nations Office on Drugs and Crime (UNODC) as "substances of abuse, either in pure form or in preparation, which are not included in the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a threat to public health". The term "new" does not necessarily refer to the recent discovery of these substances, as many of them were synthesised decades ago, sometimes for pharmaceutical research purposes that were not commercialised and are now emerging as recreational drugs.

Their legal status may vary from country to country. In Argentina, many of these substances were included in Annex I of Decree 560/2019. However, for those substances that are not explicitly named, Annex II allows for their regulation based on their chemical structure.

To date, the UNODC's Early Warning Advisory (EWA) has reported 1300 new psychoactive substances (NPS), of which 34% are classified as stimulants. Synthetic cathinones constitute a population of more than 221 reported substances within this category [1]

Synthetic cathinones are chemical compounds derived from Cathinone; a natural alkaloid extractable from the Khat plant (Catha edulis). It is a β-keto analog of amphetamine and shares its phenethylamine structure, as well as the stimulating effects it induces in the body [2].

Methylone was the substance that initiated reports of synthetic cathinones, first appearing in 2005 to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), marketed mainly online under the name "Explosion" [3,4]. Historically, synthetic cathinones were marketed using labels that facilitated regulatory evasion, with inscriptions such as "bath salts," "not for human consumption," "research chemicals," etc. In some countries, these products were available in physical stores, gas stations, smoke shops, etc., in the early

2000s but following the regulation of these various cathinones, their commercialisation primarily occurs through the internet (darknet or crypto-market)[5]. Currently, synthetic cathinones are sold disguised as "ecstasy" tablets, leading many MDMA consumers to be unaware of the inclusion of synthetic cathinones. N,N-Dimethylpentylone (Dipentylone or Dimethylpentylone) was first identified in Sweden in 2014 [6] and has been reported to the EWA system 153 times to date [7]. Although an operation linked to N,N-Dimethylpentylone was recorded in Argentina in 2022 no analytical data supporting the finding were published.
The aim of the present communication is to delineate the analytical procedures and the results obtained from the identification of N,N- Dimethylpentylone in a solid sample submitted to the Toxicology and Forensic Chemistry Laboratory of the Judicial Investigations Corps, which is part of the Public Prosecutor's Office of the City of Buenos Aires. This laboratory is responsible for the analysis of seized samples in drug-related judicial proceedings in the City of Buenos Aires.

Material and Methods

The sample analysis was carried out by the Toxicology and Forensic Chemistry Laboratory of the Judicial Investigation Service of the Public Prosecutor's Office. The sample was presented as a pink tablet, in the shape of a shield, with the distinctive “Ferrari” logo on one of its sides, with a net weight of 521.3 mg. The tablet presented heterogeneity in its composition, with small crystals observed throughout the sample.

A pink shield-shaped tablet bearing the distinctive :Ferrari” logo
Net weight: 521.3 mg
Measures: 0.7x0.8 mm

According to protocol, all pills entering the Toxicology and Forensic Chemistry Laboratory are subjected to a Marquis reagent test. A portion of the sample was homogenised for subsequent analysis.
Some of the crystals observed inside the pill were carefully separated for analysis by Fourier Transform Infrared Spectroscopy (FTIR) using a Perkin Elmer Two model instrument under the following conditions:

Table 1: FTIR conditions used

System of Analysis	Attenuated Total Reflectane (ATR) with diamond glass
Analysed Spectral Range	4000-650 cm^{-1}
Acceptance Requirements	A coincidence index higher than 0.90 and the peaks obtained in the fingerprint region do not differ by more than +/- 3 cm^{-1} from the instrument’s database

Simultaneously, a representative fragment of the tablet was homogenised, selecting 40 mg and subjecting it to three extractions with 1.5 mL of HPLC-grade methanol, isolating the insoluble fraction. Subsequently, it was analysed by FTIR under the same conditions mentioned in Table I.
Then, 20.0 mg of the sample was homogenised with a porcelain mortar and pestle and 2.0 mL of HPLC grade methanol was added. The solution was thoroughly homogenised with a vortex mixer and centrifuged at 3000 rpm for 3 minutes. 75 µL of the previous methanolic fraction was extracted and placed in a chromatography vial, together with 125 µL of 250 ppm tetracosane solution (internal standard) and 1300 µL of HPLC grade methanol.
The sample was analysed using the Gas Chromatography-Mass Spectrometry technique under the following conditions:

Table 2: GC-MS Conditions used

Equipment	Shimadzu Heals 2030 gas chromatograph coupled to a single quadrupole mass spectrometer (Shimadzu Nexis 2030) with an electron ionization mode system
Column	Shimadzu SH-5MS model, 30 m length, 0.25 mm ID and 0.25 µm film thickness
Injection	1 µL injection in Split mode (1:30), using an AOC 20 I•PLUS automatic injector with 20S-U autosampler, employing Helium 5.0 as carrier gas. The injection temperature was 280°C
Oven conditions	The Initial column temperature was 120°C maintained for 5.0 minutes, then increased to 220°C at a rate of 25.0°C/min. Finally, it was increased to 280°C at a rate of 75 °C/min with a final isothermal period of 3 minutes
System of Analysis	Detector Interface temperature set at 280°C; Ion source with Electron Ionization (EI) mode at 70 eV and a temperature of 230°C
MS Parameters	Scanned in SCAN mode with a solvent delay of 2.5 minutes and a scanning range of 40-450 amu at 0.20 scans/second

Finally, a complementary analysis was carried out using a more selective methodology to reinforce the results obtained by FTIR in the identification of N,N-Dimethylpentylone. For this analysis, the Microanalysis and Physical Methods in Organic Chemistry Unit (UMYMFOR) at the National University of Buenos Aires was asked to analyse the sample using an Agilent RRLC 1200 ultra-high performance liquid chromatograph (UHPLC), equipped with a Phenomenex Kinetex HILIC 100Å 150 x 2.10 mm x 2.6 µm column, coupled to a

Bruker micrOTOF-Q II quadrupole time-of-flight mass spectrometer.
10.0 mg of the previously homogenised sample was weighed and 1 ml of methanol HPLC grade was added. The solution was shaken vigorously with a vortex mixer and a 1:3000 dilution with MS grade methanol was made for introduction into the system. A solvent gradient was used with 10 mM ammonium formate adjusted to pH 3 with formic acid as Solution A and acetonitrile as Solution B, with a constant flow rate of 0.3 ml/min at 25 °C. The total run time was 20 minutes (see table III).

Table 3: LC Gradient Conditions

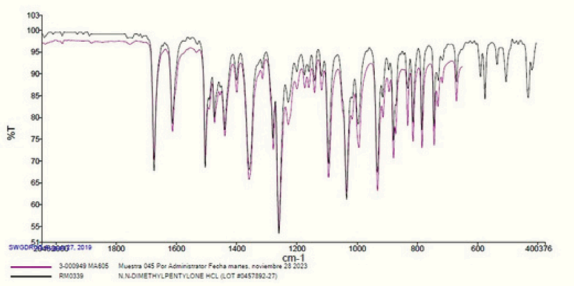
Time (min)	Solution A (%v/v)	Solution A (%v/v)
0	10	90
5	10	90
7.5	20	80
9	30	70
15	90	10
17	90	10
18	10	90
20	10	90

Initially, the sample was analysed to identify the precursor ion present and subsequently a spectral analysis was performed to obtain a fragmentation and identify the daughter ions of the molecule.

Results

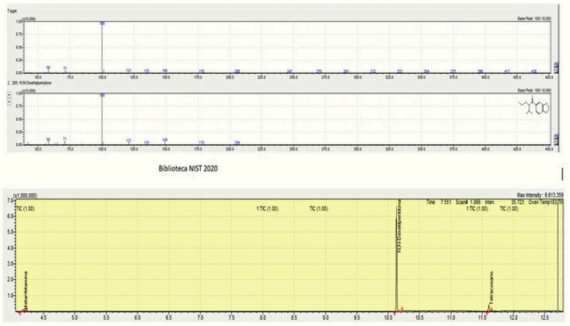
Upon contact with the Marquis reagent, the sample presented a yellowish color, unlike the positive control in the MDMA (3,4- Methylenedioxymethamphetamine) assay, which is the most frequently identified amphetamine in our laboratory. In the FTIR analysis enabled the identification of N,N-Dimethylpentylone based on spectral comparison with the instrument's database, which includes the SWGDRUG 2.1 (2019) IR spectrum library, showing a high degree of similarity [8]. The matching index was 0.98235 for N,N-Dimethylpentylone HCl. In this regard, the overlaid spectra (sample and database) revealed the coincidence of characteristic peaks: 1674, 1614, 1504, 1439, 1360, 1261, 1095, and 933 cm^{-1} (See Figure 1).

Figure 1: Comparative FTIR Spectra



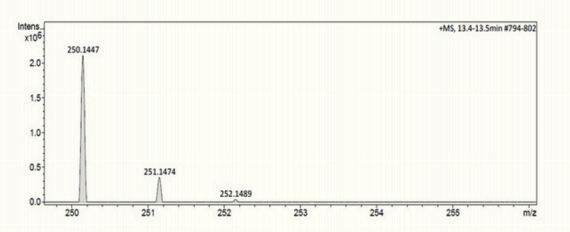
Additionally, the obtained spectrum was compared with structural isomers of the substance, revealing differences with spectra such as N-Ethylpentylone [9] or Tertylone. This allowed the identification of the substance in question both in the crystals extracted from the pellet and in the solution obtained by homogenization and dissolved in methanol. Likewise, the methanol-insoluble fraction was analyzed under the same FTIR conditions and microcrystalline cellulose was identified.
Analysis through Gas Chromatography Mass Spectrometry (GC-MS) coupled with single quadrupole mass spectrometry enabled the identification of m/z fragments corresponding to methamphetamine, our synthetic cathinones and tetracosane (internal standard) (see figure II). Methamphetamine was confirmed by retention time with the reference standard and analysis of the generated mass spectrum. The m/z fragments were cross- referenced with spectral libraries NIST 2020 and SWGDRUG 3.12 included in the instrument. Something to highlight is that the mass spectrum obtained is compatible with different synthetic cathinones such as N,N Dimethylpentylone, N-Ethylpentylone, Hexylone, N-Propylbutylone and 3',4' Methylenedioxy N,N diethylcathinone which have a molecular weight of 249.3 and a base peak of m/z=100 when ionised by electron impact and not all of them are included in the spectral libraries mentioned.

Figure 2: Gas Chromatography Mass Spectrometry



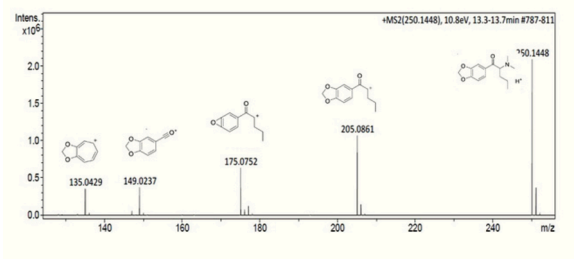
The molecular formulas obtained by liquid chromatography were assigned based on the exact mass value and isotopic distribution compared with calculated values. The analysis allowed for the identification of the [M+H]+ ion at m/z 250.1447 (calculated for C14H20NO3+ as 250.1438) (Figure III).

Figure 3: Isotopic Distribution



Above (Figure IV) is the confirmation of identity performed through MS/MS fragmentation analysis, which corresponds to N,N-Dimethylpentylone.

Figure 4: High Resolution Mass Spectrometry



The mass spectra obtained were compared using as a reference the monograph published by The Center for Forensic Science Research & Education (CFSRE)[10].

Discussion and Conclusion

The emergence of new cathinones is continuously growing and there are currently reports indicating that N,N-Dimethylpentylone predominates in the US synthetic stimulant market, which may persist for the next 1 to 2 years until a shift in consumption trends towards another substance occurs [11]. However, given the emergence of additional closely related isomeric compounds, they demand that forensic laboratories have different analytical methodologies that allow reliable identification even when there is no reference standard that allows knowing the retention time by chromatography.

Due to the lack of reference material to determine the retention time of dipentylone and considering that it has a mass spectrum with characteristics similar to some of its structural isomers, it was not possible to achieve adequate selectivity for correct identification by GC-IE-MS in our laboratory.

This is crucial when relying on GC-IE-MS as the primary or sole analytical technique, as it has limitations due to the fact that the mass spectrum obtained was compatible with different synthetic cathinones with a base peak (m/z=100). Due to the robust fragmentation that this technique possesses, it is not possible to obtain minority ions that could help in the identification of the molecule.

The Marquis reagent test yielded a result compatible with the presence of cathinone in the sample. This reinforces the importance of colorimetric screening analyses in drug seized laboratories, as they provide very important information about the possible presence of certain substances in the sample.

The FTIR technique demonstrated adequate selectivity for the identification of N,N-Dimethylpentylone, requiring the initial isolation of the crystals of the substance found within the tablet or a solution of the homogenized tablet.

Liquid chromatography with high-resolution mass spectrometry allowed an adequate identification of the substance with results compatible with those reported by The Center for Forensic Science Research & Education (CFSRE) [10].

It is important to highlight that obtaining fragments m/z= 175.0752 and 205.0861 allowed us to rule out the presence of synthetic cathinones such as 3',4' Methylenedioxy N,N diethylcathinone, N Propylbutylone and Hexylone for which we did not have infrared spectra available and the GC- MS-EI does not have the adequate selectivity to discriminate them.

Finally, it is important to highlight the importance of having different analysis techniques that allow for adequate identification of seized substances due to the increase in structural isomers that are available. Currently, Decree 560/2019 was updated to include N,N- Dimethylpentylone in its Annex I.

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Criteria For Interpretation of Postmortem Toxicological Results

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Keywords: Interpretation, Postmortem Results, Criteria, Postmortem Stability, Postmortem Redistribution, Polydrug Intoxication, Postmortem Forensic Toxicology

Introduction

Toxicology is the study of the adverse effects of drugs, poisons, and other chemicals on biological systems. Forensic toxicology is the application of toxicology to issues and cases where those adverse effects may have medico-legal consequences, with results used in court. The forensic toxicologist employs the disciplines of toxicology, analytical chemistry and pharmacology to detect, quantify and interpret drug findings in a wide variety of biological specimens. The specific role of the forensic toxicologist varies slightly depending on the specific discipline. Specifically, postmortem forensic toxicology investigates the absence or presence of drugs, both abused and therapeutic, and their metabolites, alcohol and other volatile toxins, carbon monoxide and other gaseous toxins, as well as any other chemical product (metals, pesticides, etc.) in human fluids and tissues, evaluating their determining or contributing role in the cause and etiology of death [1,2].

The interpretation of toxicological results is one of the most difficult tasks in postmortem forensic toxicology, its objective being to determine whether toxins have played any role in the cause of death [3]. It is necessary to know the details of the scene of the events, the history of the case, the autopsy findings, and their relationship with the toxicological results based on the analysis of a sufficient number of samples and performed with the appropriate analytical techniques.

Drug identification and quantification are typically performed in blood, urine, or tissue samples [1,2]. The ASB (Academy Standards Board) standards in forensic toxicology, developed by the American Academy of Forensic Sciences (AAFS), provide a framework for ensuring the quality and consistency of analytical methods and reporting in the field: method validation, reporting, and best practices for opinions and testimony [4].

The forensic toxicologist's interpretation of these results helps with the medical examiner's or coroner's determination of the cause and manner of death. Among the samples usually taken at autopsy, peripheral blood is the most important object of quantification since what is detected in it may be indicative of the therapeutic, toxic or lethal effect produced on the individual. Additionally, the use of a second sample for confirmatory practice is highly recommended [4-16]. Unfortunately, the concentrations of drugs detected in postmortem blood do not necessarily reflect the antemortem concentrations, since they can vary depending on the anatomical origin of the sample and postmortem changes. These variations in postmortem concentrations dependent on place and time are known as "postmortem redistribution" [17-31]. The stability of the detected drug, both considered by itself, and contained in the postmortem sample where it is found, is another factor, dependent on time, temperature and other environmental aspects, which must also be taken into consideration to interpret the postmortem toxicological results [32- 42].

There are tables in the literature and toxicology books that contain data on therapeutic, toxic and lethal concentrations of drugs in "blood" [43-48]. Some of them contain, without any differentiation, data from living and dead subjects, cardiac and peripheral blood, and serum/plasma and blood concentrations. The therapeutic and toxic concentration ranges sometimes appear overlapped and the pharmacological aspects, crucial to assess toxicity, are ignored. They do not take into account the cases of polydrug use, so common in our routine cases, in which alcohol, drugs, psychotropic drugs, along with

other medications or chemical products are co-administered without taking into account the potentiation of toxic effects [49-56].

Based on all of the above, the international committees of experts in forensic toxicology emphasise how risky and dangerous it can be to interpret toxicological results after a mere "isolated" comparison of the concentration of the drug in blood with the data in the aforementioned tables [57].
Regarding the concentrations of abused drugs in blood, it should be emphasised that in no case can the degree of toxic effects in the individual be inferred from them, and the tolerance phenomena frequently developed in drug addicts cannot be either ignored. In the routine practice, it is observed by toxicologists that similar concentrations of drugs of abuse (e.g. cannabis and cocaine) belong to both living and dead subjects. Abused drugs are not pharmaceuticals, and therefore there are no "safe" and/or toxic/lethal therapeutic concentrations for them. Thus, in the absence of signs of violence or trauma in the corpse, the mere presence of abused drugs justifies the cause of death by adverse reaction (drug overdose deaths) [58-60].

Objective

The objective of this work, based on all of the above, is to consider the most notable recent developments published on the redistribution and stability of drugs in postmortem samples, as these are, among others, key aspects for interpreting the toxicological findings of the corpse [17-42]. And, in addition, it is crucial to consider the cases of co-administration of different abused drugs and therapeutic drugs with potentiation of toxic effects [49-56]. To communicate all this knowledge to the scientific community contributes to the quality of expertise and to the advancement of unity of scientific criteria, key aspects in forensic toxicology.

In the last 25 years, internationally renowned scientific teams in forensic toxicology, led by Drummer, Byard, Butzbach, Pelissier-Alicot, Moriya, Logan, McIntyre, etc., have taken over the efforts initiated in the late 1980s by those led by Sunshine, Prouty, Anderson, Pounder, Levine, Caplan, Dalpe-Scott, etc., and have continued to undertake interesting studies on the redistribution and stability of toxins in postmortem samples.
From the review of new developments on alcohol redistribution in postmortem samples, the findings of the Pelissier-Alicot research group stand out concluding that when femoral blood is not available at autopsy, subclavian blood is a good alternative for postmortem alcohol determination [23, 24].
These authors also state that if death has not occurred with major trauma to the thoraco- abdominal region, blood from the right heart cavity may also be valid, although not preferred, for postmortem alcohol measurement, since the postmortem redistribution effects can affect other drugs and metabolites. After this review, it is worth emphasising the internationally accepted recommendation to use the vitreous humor to confirm the presence of alcohol in the blood, as it allows us to determine whether this comes from antemortem ingestion, or on the contrary, it is due to postmortem generation by putrefaction or contamination by major trauma [61]. From the review of new publications on drug redistribution in postmortem samples, the studies carried out by Drummer's research group on antidepressants and antipsychotics stand out, revealing that distribution initiates during the stages closest to the time of death. Therefore, it is recommended to carry out the initial toxicological scans on blood from the central cavity, as this is where, in general, the drugs are most concentrated and not subject to extensive metabolism as it occurs in urine. If the concentration in the central blood is in the toxic range, quantification in peripheral blood will clarify the existence of postmortem redistribution, and in these cases it is highly advisable also evaluate the concentrations in other autopsy samples [4].

From the review of new publications on drug stability in postmortem samples [32-42], the findings about some of them, i.e. olanzapine, indicate that molecules with ester groups, sulfur atoms, or easily oxidizable or reducible structures are preferred targets for degradation. Since the 1990s, it was found that cocaine [32], heroin, and morphine glucuronides [35,36], were examples of drugs with postmortem instability, more modern findings add pesticides [35], benzodiazepines [37], certain antipsichotics [39] and cathinones [40-42], to the list.

These studies [39], also emphasise the importance of carrying out toxicological analyses as soon as possible after the autopsy, although sometimes this is not possible given constraints in staff, equipment, and caseload. As a consequence, is highly recommended keeping the samples frozen at least at -20°C to avoid any degradation, warning that the toxicological results of samples stored for some time at room temperature should be taken with caution, given the possibility of partial degradation of the drugs.

Conclusion

In conclusion, having a thorough understanding of the phenomenon of postmortem redistribution [17-31], of which, the mechanism has not been explained, and stability of toxicants in postmortem samples [32-41], and taking into account the enhancement of toxic effects by co- administration of alcohol, drugs of abuse, psychotropic drugs and other medications or toxic agents [49-56], is crucial for the forensic toxicologist to be able to undertake the interpretation of toxicological results. This will serve as a basis for the forensic pathologists, medical examiners, and coroners to later establish, with the greatest possible veracity, the role of the drugs in the cause of death.

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Dried Matrix Microsampling for a Reliable Approach to Workplace Drug Testing

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Keywords: Workplace Drug Testing, Drug Abuse, Dried Matrix Spot, Dried Blood Spot, Dried Urine Spot, Dried Oral Fluid Spot

Drug abuse has become a serious global issue in recent decades, posing a significant burden on society. The estimated number of people who have used illicit substances in the past year continues to rise, reaching 292 million worldwide in 2022 [1] and, clearly, most people who use drugs do also have a job. Indeed, alarming evidence from European rehabilitation centers shows that a high percentage (41%) of patients are regular workers.[1]
Workplace Drug Testing (WDT) was introduced in the USA in the 1980s to detect the use of psychoactive substances in certain job settings, in accordance with national occupational health and safety regulations. Progressively, WDT was set up in several European regions.[2] The core of WDT is the preservation of a drug-free workplace to reduce economic and social risks and to enhance productivity.[3]
Currently, there are various factors that differentiate WDT rules across countries [4], including:
• the category of job subjected to monitoring;
• the frequency of testing;
• the timing of analysis (pre-employment, random, post-incident and other specific situations).

The workflow generally consists of an initial screening test, typically carried out with immunological assays, followed by confirmation by means of gas or liquid chromatography coupled with tandem mass spectrometry. In terms of biological specimens, urine has been the gold standard matrix since the first regulations were [5] enacted.

Toxicological and clinical research has focused on innovative strategies for collecting and analyzing biological fluids to streamline the pre-analytical phases, namely sampling, transportation and storage. In this context, blood microsampling, which involves capturing small drops of capillary blood (<100 µL) onto cards, has emerged as a viable alternative to traditional vascular puncture, significantly advancing human healthcare[6]
The drying step after microsampling action ensures long-term stability of molecules, preventing degradation caused by time and temperature factors and rendering pathogens inactive, thereby mitigating biohazard risks [6].
These concepts led to the pioneering Dried Blood Spot (DBS) technique in the early 1960s for diagnosing neonatal metabolic disorders [7]. Over time, this technique has expanded into various fields, including therapeutic drug monitoring, disease biomarker research, forensic toxicology, and anti-doping analyses in sport. [8]
It is noteworthy to highlight that WDT has not been in depth explored yet as a possible field of application for DBS. To date, while the use of hair matrix is on rise, urine and oral fluid remain the most commonly used biological sample for WDT. DBS microsampling could be effective for workers and employees undergoing toxicological analysis, although in most countries the related laws or regulations have not considered the DBS option yet. DBS belongs to Dried Matrix Spot (DMS) systems, and it is by far the most widely used, largely because it was the first technique to be developed. Recently, other biological fluids, such as oral fluid and urine, have also been adapted to the dried spot approach, obtaining respectively Dried Oral Fluid Spot (DOFS) and Dried Urine Spot (DUS) microsamples. [9]

The use of specifically designed volumetric devices (such as Capitainer B, Hemaxis, HemaPEN, VAMS) [10] to collect and dry precise volumes of blood, urine, or oral fluid presents practical advantages for WDT. Minimal invasiveness allows for a painless self-sampling, which can occur directly at the workplace

and not in health facilities, eliminating the need for stringent transport conditions typically associated with liquid samples. During the sample preparation, straightforward methodologies are carried out to extract the molecules from the dried biological matrix.

These features open up the possibility of more frequent sample collection, potentially contributing to a comprehensive approach for addressing drug consumption. Indeed, mainly due to the minimally invasive nature of sample collection, the DMS approach is preferred over the corresponding liquid matrix in other contexts such as the TDM in clinical settings; likewise, workers may be more inclined to provide samples, allowing large-scale screening at minimal costs and logistics, similarly to participants in longitudinal clinical trials. [11]

The primary goal of WDT is to detect the recent use of drug, rather than addiction. The flexibility of DMS techniques directly addresses the needs of WDT [3], to various analytical detection methods. Whether using immunological assays or tandem mass spectrometry, the DMS protocols could be optimized generating quick, cost-effective and efficient tests, with a peculiar regard to sensitivity, which is a crucial parameter within toxicology. DMS samples have volumes 10 to 50 times smaller than traditional liquid biospecimens [12], issue in terms of sensitivity. However, recent advances in tandem mass spectrometry technology allowed for low limits of detection and quantification, fulfilling the cut-off requirements set by official guidelines [8].
In the context of WDT the cut-off approach is generally applied for determining whether a subject is considered as positive in terms of being impaired or simply having previously used drugs.

Particular attention should be given to the choice of the device used for microsampling. Typically, it consists of a filter paper that absorbs the liquid matrix and ensures its drying within an average time of three hours [13]. One challenge encountered in DBS practice was the difficulty in obtaining a valid biospecimen due to variability in blood drop spread, which in turn is influenced by hematocrit levels [14]. Moreover, DMS on the standard card cannot provide accurate measurements due to its non-quantitative nature. For this reason, new microsampling technologies has evolved to achieve a precise volume. These devices have been initially designed specifically for DBS, but now they are also employed for collecting oral fluids and urine, or the manufacturing companies provide the appropriate device tailored to the specific matrix.
In the context of WDT, the choice of the DMS may vary depending on the specific case. It is widely acknowledged that a perfect sample does not exist, but it is considered good practice to appoint the ideal matrix for the specific purpose. It is advisable that guidelines are soon drafted, outlining priorities and recommendations for each step of the analytical workflow to combine regulations and scientific aspects.

WDT applications for DMS are diverse [5]. A realistic example of a real-world scenario is where industries or institutions need to assess the impairment of workers caused by recent drug use through random testing. Again, current employment law mandates drug testing based on the analysis of traditional drugs of abuse in urine or oral fluid in most cases[15], while the introduction of DMS remains advisable.

DBS and DOFS provide a detection window for the past 24 hours, while DUS may be used to evaluate a longer period, in addition to the possibility of quantifying drug metabolites and exploring possibly metabolic and kinetic aspects with the reference to dose consumption.[16]
The post-accident situation represents a second instance where drug testing is required. The DOFS sample could be collected more promptly than DBS owing to greater simplicity of oral fluid sample, and it can provide information about recent drug consumption prior to the incident. [17]

Regarding the toxicological analysis to job applicants, the DUS test can be more useful than other DMS because it is able to reflect a temporal spectrum up to 3-5 days before the sample collection.
Therefore, the DMS sampling method would enable the implementation of a workplace monitoring program, facilitating the microsampling of blood, urine or oral fluid at fixed intervals. Guidelines or specific regulations would be needed.
The rationale behind this strategy can be seen as a response to the increasing drug use among workers, making the prevention of injuries and accidents an urgent priority. The DMS described could be combined to enhance the interpretation of results, rendering complementary the dried matrices.
Overall, in a field like Workplace Drug Testing, which is marked by a variety of nuances in legal and scientific terms between countries in Europe and America, the DMS microsampling approach can be emphasised as an effective methodology. It reinforces the entire analytical process while significantly minimising the discomfort for individuals being tested [9].

Acronyms
Workplace Drug Testing (WDT)
Dried Blood Spots (DBS)
Dried Matrix Spot (DMS)
Dried Oral Fluid Spot (DOFS)
Dried Urine Spot (DUS)
Volumetric Absorbitive Microsampling (VAMS)
Therapeutic Drug Monitoring (TDM)

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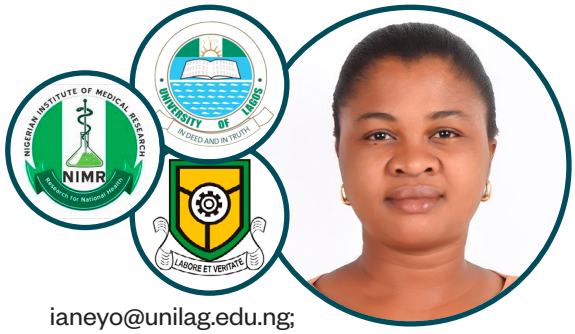

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Nigeria's Drug Crisis and Youth Vulnerability from an African Perspective on Local Concoctions

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Keywords: New Psychoactive Substances, Illicit Substances

Introduction

Illicit drug use in Africa, particularly Nigeria, has shifted beyond traditional narcotics like heroin and cocaine, evolving into a complex crisis shaped by socio-economic, cultural and political factors [3]. The World Drug Report 2023 (UNODC) underscores that cannabis continues to be the most widely consumed drug globally, with its potency steadily increasing, while synthetic opioids, notably fentanyl, are driving unprecedented levels of overdose mortality. At the heart of this shift is the rise of novel psychoactive substances (NPS) a diverse class of plant-based, synthetic, or unconventional materials (including animal remains) designed to mimic the effects of drugs such as cannabis, cocaine, ecstasy ("Molly"/MDMA/ 3,4-methylenedioxyamphetamine), 3,4-Methylenedioxyamphetamine (MDA) and LSD (Lysergic Acid Diethylamide), while often evading detection and regulation; [2, 4]. In Nigeria, the spread of locally produced NPS like "Gutter Water," "Zakami," and "Monkey Tail" has worsened the public health crisis, posing major challenges to healthcare and law enforcement [2]. These drugs are easily accessible and affordable, especially among youth and the unemployed. Polydrug use mixing substances like tramadol or codeine with alcohol is common, heightening the risk of overdose and long-term harm[5]. Drug-related aggression, withdrawal, and family conflict are widespread, reflecting broader societal impacts [2]. Rooted in poverty, disenfranchisement, and systemic neglect, this crisis demands urgent, culturally sensitive and context-specific responses. Yet, despite growing concern, there remains a critical research gap in the analytical profiling and chemical characterization of locally consumed NPS in Nigeria. Existing studies largely address usage patterns and socio-economic factors [5], with few focused on the scientific identification and pharmacological properties of these substances. Without this knowledge, effective clinical care, harm reduction, and legal control are severely limited.

Methodology

This study analyzed 150 valid responses collected via a structured questionnaire aimed at evaluating awareness, usage patterns, risk perceptions, and social dimensions of illicit drug use across Nigeria. The data collection employed convenience sampling, targeting a general adult population. Questions ranged from socio-demographics to personal experiences and beliefs about illicit drug use. Quantitative and qualitative items were included (A blank form is included below the references). Statistical analysis was performed using Python, including descriptive analysis, reliability testing via Cronbach's alpha and a qualitative risk assessment.

Ethical Statement

Ethical approval was obtained from the University of Lagos ethical committee and approved.

Results and Discussion

Demographics, Awareness and Usage Patterns

The study sample comprised individuals of diverse genders, age groups, educational levels and occupations who told us that they used either or both

conventional or non-conventional psychoactive substances thus, they were aware of illicit and psychoactive drugs, with marijuana, tramadol and cocaine among the most commonly recognized types. Sources of drug-related information were predominantly informal peers, social media and movies highlighting the strong influence of non-institutional channels. About one-third of respondents admitted to using illicit drugs, typically initiating use in their late teens to early twenties. Peer pressure and curiosity were leading motivators. Marijuana, tramadol and codeine were the most frequently used substances, with some users mixing them with alcoholic or non-alcoholic beverages to intensify effects or manage emotional distress.

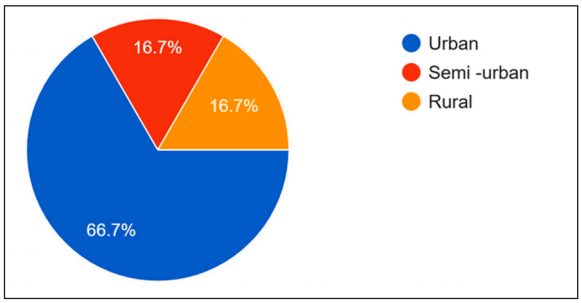
Perceived Risk and Access

When rating their knowledge of drug risks, most participants described it as "moderate" to "high." However, internal consistency among these risk perception items was poor. Cronbach's alpha (a statistical method for analyses for analysis Likert-style items), calculated from Likert-style (a rating scale with five or more options used to measure opinions, attitudes, or behaviors.) items (knowledge of risk, ease of access, and perceived seriousness), was 0.63, indicating unreliable internal consistency likely due to low correlation among these variables or poor item construction.

Risk Analysis

In urban areas as shown in Figure 1, youths and the unemployed are most at risk, as drugs are easy to access and widely normalized. The consequences go beyond health, leading to aggression, withdrawal, and family conflict. With polydrug use common, the risk of overdose and long-term harm is especially high, making drug use a pressing public and community concern.

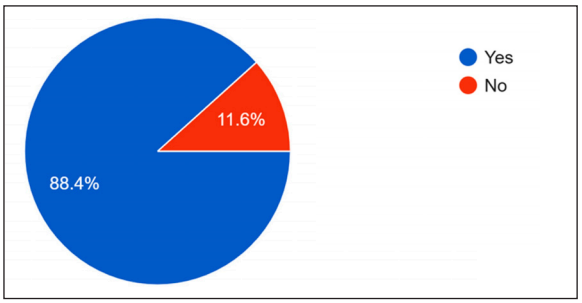
Figure 1: Area of Residence Respect to Urbanisation



Social Dimensions

Peer pressure was a major driver of drug use. As shown in Figure 2, 88% of participants acknowledged that peer influence plays a role in substance use. When asked how they first encountered drugs, 95% (129 respondents) traced it to social settings through friends, parties, clubs, secondary schools, or older siblings. This highlights how deeply social environments shape initiation into drug use.

Figure 2: Social Influence on Consumption of Drug Addiction



Regional Variation in Psychoactive Drug Use

Literature indicates that patterns of psychoactive substance use in Nigeria vary significantly across regions, shaped by cultural norms, economic conditions, religious influences, and substance availability. Based on secondary data, a risk index map (Figure 3) was created, ranging from Low [4] to Critical[9], to capture these regional differences. The North-East shows higher prevalence of inhalants and zakami, the South-East and South-South are associated with concoctions such as Monkey Tail and Gutter Water, while the South-West is marked by synthetic drugs like Colorado and pharmaceutical mixtures (Table 1). These findings highlight that the risks of drug use are context-specific and rooted in local realities, underscoring the importance of regionally tailored responses.

Figure 3: Map of the Geographic Distribution and Risk Index of Illicit Drug and Psychoactive Substances in Nigeria

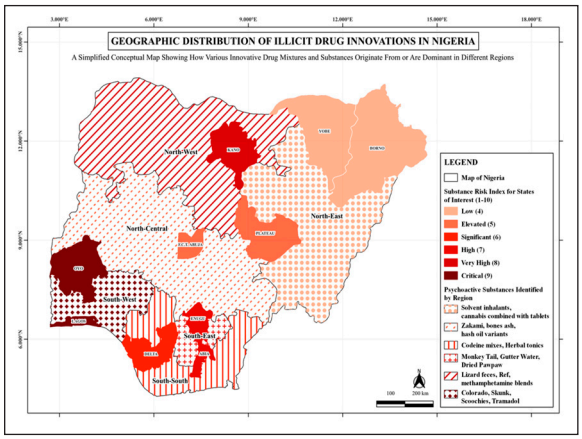


Table 1 highlights the blend of imported and locally formulated substances, reflecting how young users identify and misuse these drugs based on availability, subcultural knowledge or desired psychoactive effects. Notably, concoctions such as "Scoochies" or "Codeine Cough Syrup Mix" involve multiple substances (cannabis, alcohol, tramadol) and often evade standard regulation. Survey responses indicate signs of substance use disorder among some participants, including repeated use and polydrug mixing. Many reported using drugs to cope with stress, suggesting co-existing mental health issues. Adverse effects such as aggression, withdrawal symptoms and family conflict were common. The normalization of drug use

Table 1: Market Names and Composition of Common Psychoactive Substances Used in Survey, 2024

Market Name	Local Nigerian Name(s)	Local Understanding of Active Ingredient	Scientific Name (Active Ingredient)
Canadian Loud	Loud, Igbo, Ganja	Strong/high weed	Tetrahydrocannabinol (THC)
Molly	Molly, Mandy	Party/Euphoria pill	MDMA (3,4-Methylenedioxymethamphetamine)
Refnol	Roche, Ref, Blue Pill	Sedative/'Knockout' drug	Flunitrazepam
Ice	Ice, Crystal, Glass	Stimulant/'Brain booster'	Methamphetamine
Tramadol	Tramol, TEE	Painkiller/misused for energy or sex	Tramadol Hydrochloride
Colorado	Colos	Synthetic weed; causes hallucination	Synthetic Cannabinoids
Cocaine	Cokie, White Sugar	High-end stimulant	Benzoylmethylecgonine
Skunk	SK, Loud, Ganja SK	Potent weed hybrid	High-THC Cannabis Hybrid
Rohypnol	Ref, Roche	Sedative/'Date rape' drug	Flunitrazepam
LSD	Acid, Tabs	Hallucinogen/'Spiritual drug'	Lysergic Acid Diethylamide
Scoochies	Monkey Tail, Igbo soup	Weed boiled in gin or herbs	THC (from Cannabis) + Alcohol
Codeine Cough Syrup Mix	Purple Drank, Zobo Mix, Eja Osan	Codeine, tramadol, and alcohol in zobo/juice	Codeine, Tramadol, Ethanol
Zikami	Zikami(common in Northern Nigeria)	Plant Based Ingredient	*Datura metel* (tropane alkaloids)
Monkey Tail	Monkey tail	Concoction of local gin mixed with *Cannabis sativa*	Unknown

and its accessibility raise significant public health concerns.

Conclusion

Illicit drug use in Nigeria has evolved into a multifaceted public health crisis, driven by locally concocted substances from both plant-based and synthetic sources. Young adults (20–30 years) remain particularly vulnerable, with polydrug use normalized through peer influence, socio-economic hardship, and easy access. This has intensified health risks and contributed to broader social instability. The World Drug Report 2023 underscores the urgent need to integrate mental health services into drug policy responses. Reinforcing this perspective, Nigeria must adopt culturally responsive and evidence-based strategies such as youth-focused education, scientific profiling of substances, and regional-specific interventions to address both psychosocial and clinical dimensions of drug use. Tackling root causes while embedding mental health into policy frameworks is essential to building resilience and ensuring long-term public safety.

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Example of Survey

Questionnaire for Illicit Drugs
Section 1 of 5

Prevalence of Illicit Drugs
The prevalence of illicit drugs among youths. Your participation will provide valuable insights into the patterns and frequency of drug use, aiding in the development of effective prevention and intervention strategies. Rest assured, your responses are confidential and will only be used for research purposes. Thank you for contributing to this important study to improve youth health and safety.

1. Do you agree to participate in this survey?
o Yes
o No

Section 2 of 5

DEMOGRPAHIC INFORMATION
2. Age
o 10-20 Years
o 21-30 Years
o 31-40 years
o 41-50 Years
o 51 Years and Above

3. Gender
o Male
o Female

4. Marital Status
o Single
o Married
o Divorced

5. Educational background
o Primary
o Secondary
o Tertiary

6. Occupation _____
7. Religion _____

8. Area of residence
o Urban
o Semi-urban
o Rural

9. Living arrangement
o Alone
o With parents/guardians
o With spouse/partner
o With friends
o Other...

Section 3 of 5
KNOWLEDGE AND AWARENESS
10. Have you ever heard of illicit drugs?
o Yes
o No

11. Which of the following do you consider illicit drugs (Select all that apply)
o Cannibis
o Cocaine
o Heroin
o Tramadol (abused)
o Codeine (without prescription)
o Canadian Loud
o Molly
o Refnol
o Ice
o Colorado
o Skuunk
o Rohypnol
o LSD
o Scoochies
o Other

12. What are common sources of information about drugs for you? (Select all that apply)
o Friends
o Social Media
o Television/ Radio
o School
o Religious Centers

o Health Professionals
o Other...

13. How would you rate your knowledge of the risks of drug abuse?
o Poor
o Fair
o Good
o Excellent

Section 4 of 5
DRUG USE PATTERN
14. Have you ever used any illicit drug?
o Yes
o No

15. If yes, at what age did you first use it?_____

16. How did you first become introduced to illicit drugs? __

17. Which drugs have you used? (Select all that apply)
o Ghana loud
o Canadian loud
o Molly
o Rohypnol (Ref)/Sweet /542
o LSD (Lysergic Acid Diethylamide)
o ICE (Methy)
o Tramadol
o Colorado
o Cocaine
o Skunk (SK)
o Renol
o Scoochies
o Codeine Cough Syrup Mix
o Other...

18. How frequently do you use these drugs?
o Rarely
o Frequently
o Occasionally

19. Why do you use this drug(s)?
o Recreational purposes
o Curiosity and Experimentation
o Self-medication
o Social and Cultural factors
o Escapism
o Peer pressure and social influence
o Availability and Accessibility
o Improve assimilation
o Other...

20. Where do you usually get drugs from (if applicable)?
o Friends
o Street Vendors
o Online Platforms
o Pharmacies (without prescription)
o Other...

21. How easy is it to access illicit drugs in your area?
o Very easy
o Easy
o Difficult
o Very Difficult

Section 5 of 5
EFFECTS AND PERCEPTION
22. Have you experienced any health or social problems due to drug use?
o Yes
o No

23. If yes, what type of problems? (Select all that apply)
o Health complications
o Loss of job/income
o Academic failure
o Arrest/imprisonment issues
o Family/relationship issues
o Other...

24. Which of these drugs do you consider most potent?
o Cannabis
o Tramadol
o Codeine
o Methamphetamine
o Cocaine
o Others: ____

25. What experience do you typically experience after using these drugs?_____

26. Have you ever tried illicit drugs as a form of self-medication or to cope with stress or emotional issues?
o Yes
o No

27. Do you think peer pressure plays an important role in influencing drug addiction or dependency?
o Yes
o No

28. Are you aware of individuals mixing these drugs with alcoholic or non-alcoholic drinks?
o Yes
o No

29. If yes, which drugs are commonly mixed?
_____

30. Why are the drugs mixed? _____

31. Have you ever witnessed or experience any adverse effects from mixing these drugs with drinks?_____

32. Do you think illicit drug use is a serious problem in your community?
o Yes
o No

33. Do you know anyone personally affected by drug abuse? (Select all that apply)
o Yes
o No

34. What do you think is the best way to reduce illicit drug use? (Select all that apply)
o Awareness campaign
o Strict laws
o Rehabilitation services
o Family/faith-based support
o Youth empowerment programs
o Other...

35. In your opinion, which group is the most at risk of drug abuse in your community?
o Teenagers
o Young adults
o Unemployed individuals
o Student
o Other...

36. Is there anything else you would like to share about your experience on illicit drugs? _____

Commentary: Is Metabolomics a Useful Tool for the Evaluation of the Emerging Group of "Nitazene" 2-Benzylbenzimidazole Synthetic Opioids

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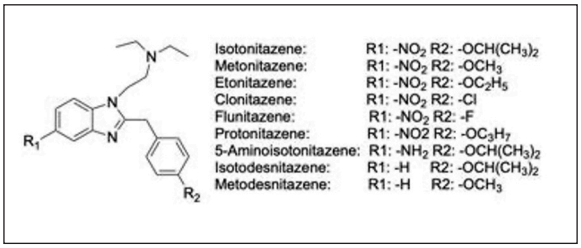
Keywords: Nitazenes, Metabolomics, New Psychoactive Substances, 2-benzylbenzimidazoles

New psychoactive substances (NPS) are compounds that mimic the effects of classic illicit drugs of abuse [1]. They have been defined by the United Nation office on Drug & Crime (UNODC) as "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat"[2]. There have been seven new synthetic opioids notified to the EU Early Warning System in 2023, six among them belong to the highly potent group of benzimidazole opioids (nitazenes).

There have been at least 20 EU countries who reported the emergence of nitazene since 2019 [3]. More recently, 5-methyl etodesnitazene has emerged in the drug market in 2024 [4]. N-Pyrrolidino isotonitazene was identified by CanTEST in September 2024 and later drug checking services in Australia have identified it in October 2024 [5]. Latvia and Estonia reported increased deaths resulting from nitazenes consumption in 2023 [3]. Products of the street have been mis-sold as heroin [3] or benzodiazepines, and cannabis products [6] but nitazenes can be far more potent than heroin, typical street doses pose highly lethal risks [3]. Nitazenes or 2- benzylbenzimidazole opioids (BO) were first synthesized in the 1950s by pharmaceutical companies as potential analgesics [7-9]. The nitazenes have not been approved as they have high abuse potentiality.

These substances have relatively high risks of respiratory depression, confusion, nausea, and vomiting, coma leading to death [9,11]. They mimic classic opioids by acting on the μ-opioid receptor (MOR) as agonist. The general structure of nitazenes consists of a benzimidazole core rings substituted with an N-ethylamine side chain at position 1 and a phenylalkyl chain in position 2 [7].

Figure 1: Nitazene core structure [11]



Meanwhile their chemical structure is dissimilar to morphine and other opioids. They are considered as opioids since they have great affinity for the μ-opioid receptor [10]. While the Office for National Statistics (ONS) does not report drug poisoning deaths attributed specifically to nitazenes, it has documented a continuing rise in overall drug-misuse related fatalities. Highest number of deaths related to drug poisoning in England and Wales have been registered in 2023 since they started recording in 1993 [12]. A number of fatalities, approximately 179 associated with nitazenes across England were reported by The Office for Health Improvement and Disparities (OHID) between 1st of June 2024 until 31st of May 2024 [13].

Protonitazene remains the most prevalent nitazene, followed by metonitazene [14]. In general, major analytical challenges associated with NPS may be attributed to the lack of understanding of their chemical composition and potential similarity to other NPS. There is a lack of reference material making it difficult to develop robust methods for new drugs [15]. The analytical challenges of NPS involve not only their diverse chemical structures [16], but also the rapid emergence [17], modification, and disappearance of these substances thus complicating detection efforts [16]. The drug market develops quickly, and numerous novel substances are identified annually. Additionally, the instability of many NPS compounds poses significant challenges in biological specimen analysis [18].

Recent findings have measured stability of four nitazenes (etazene, flunitazene, isotonitazene, and protonitazene) in dried blood spot (DBS) and all four nitazenes were found to have degraded severely at room temperature, the samples were initially prepared with 1 ng/ mL were undetectable after 30 days. Etazene and isotonitazene have degraded in the same pattern at 4 °C. Meanwhile, flunitazene and protonitazene were more stable at that temperature [19]. Even the stability of nitazenes metabolites is a concerning issue globally, Walton et al conducted a stability test for 9 nitazenes in a pool of blood under 4°C storage conditions and results showed samples samples were stable for 60 days except 5-aminoisotonitazene where there has been a significant loss after just 10 days and it failed criteria needed for quantitation under the refrigerated conditions [20]. Also, samples are susceptible to bacterial and fungal growth when they are not preserved and stored in ideal conditions and can form degraded products.

Examining post-mortem blood samples have suggested bacterial activity that accelerated the degradation of the parent compound metonitazene to 5-acetamidometonitazene [21]. Storage conditions, including temperature, time, type of specimen, and preservatives used, have not been extensively examined for most NPS particularly nitazenes. Another difficulty relays in conducting ethically approved studies on the effects of NPS including nitazenes in humans as the potential toxicity of many compounds is unknown. Postmortem issues, including redistribution, are not fully understood yet with many NPS. Furthermore, the frequent occurrence of intoxications involving drug combinations necessitates consideration of issues due to drug-drug interactions [18]. Blood samples are useful for detecting acute intoxication with rapidly metabolised drugs including NPS due to their short detection window [22]. Jadhav et al studied isotonitazene, butonitazene and protonitazene in vitro and incubated them in human liver microsomes (HLM), human S9 (HS9) fractions. Results showed they have been metabolized rapidly, within 60 minutes 95% of them have been depleted, and within 30 minutes of incubation with CYP2D6, butonitazene was depleted by 99%, isotonitazene by 72%, and protonitazene by 100%. Also, the clearance was high in comparison to verapamil [23]. However, the microbiome-drug metabolism axis can significantly influence the efficacy and bioavailability of other pharmacological substances.

Gut microbiota may impact drug-metabolizing enzymes. For example, cytochrome P450 enzymes (CYPs), particularly CYP3A4 which metabolizes over 60% of drugs, the liver protein expression levels of the homolog CYP3a11 were significantly lower in adult germ-free mice. Additionally, hydrolase enzymes produced by gut microbes including proteases, glycosidases, sulfatases, and esterases contribute to the breakdown and further metabolism of orally ingested substances, with a variety of protease types being dominant in different regions of the gastrointestinal tract [24]. Nitazenes identified in samples are often found to be impure [25] or mixed with other central nervous system depressants such as benzodiazepines or other opioids, particularly fentanyl [4] and heroin [26]. Polydrug ingestion is another challenge to be considered in forensic toxicology casework. As polydrug consumption may result in interactions, making it difficult to determine which drug or drugs caused a fatal outcome [27]. Earlier findings may lead to conclude that the parent drug may not be easily detected [28]. Detection of metabolite without the presence of the parent drug suggests the ingestion of that particular compound[20].

Metabolomics is the youngest member of the omics family [29]. Metabolomics is a technology that aims to analyze metabolites and other low molecular weight compounds within the biological system such as the cell, biofluid, tissues or organs [30, 31]. It is a comprehensive quantitative analysis tool of metabolites [28]. This tool can identify and quantify endogenous and exogenous low-molecular-weight (<1 kDa) small molecules or metabolites. As metabolites are the end downstream product, they can be a beneficial source of biomarkers discovery[23]. It may be a promising tool to help overcome challenges as it has been involved in many fields such as pharmaceuticals, transplant

monitoring, toxicology, ecology and early disease discovery [32-34]. Salivary analysis for the early detection of Alzheimer's disease (AD) employing metabolomics has been conducted [35]. Specifically, metabolomics approaches have been applied in the field of toxicology to screen biomarkers of new (exogenous) drug metabolites, detect endogenous biomarkers that indicate recent drug use and identify endogenous biomarkers for addiction or to evaluate the severity of intoxications [36,37]. Metabolomics has demonstrated considerable promise in forensic medicine by supporting cause and manner of death determination, estimating postmortem with intervals (PMI) and clarifying underlying fatal pathologies [28].

Metabolomics has been used to study drug metabolism, as the biotransformation knowledge is crucial. Particularly it is important tool in urine screening approach and the parent drug cannot be detected, the unique metabolites could be a confirmation of drug ingestion, usually the main focus is the major metabolite but if there are plenty of structurally relative substances, the minor metabolites may be required to definitively establish the consumption of a specific drug [20,37].

The first metabolomics study has been carried out by nuclear magnetic resonance (NMR) [38]. However, recent metabolomics researches have employed liquid chromatography followed by mass spectrometry (LC-MS) moreover than other methods [39]. Overall, Mass spectrometry (MS) is a more sensitive tool than NMR [33]. Also, according to Wishart LC-MS has superior sensitivity compared to Gas chromatography-mass spectrometry (GC-MS), has potentiality to detect the largest portion of metabolome and it is compatible with liquids and solids ,and requires no prior derivatization [34]. Liquid chromatography-high resolution mass spectrometry (LC-HRMS) enables the detection of thousands of metabolite features from biological samples with superior sensitivity and broad coverage, surpassing the capabilities of a wide range of methods. While untargeted metabolomic datasets are exceedingly complex, with file sizes often reaching gigabytes per sample due to the high resolution of instruments like Orbitrap®, Time-of-Flight Mass Spectrometry, (TOF-MS), and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS), advancements in softwares such as MathDAMP, MetAlign, MZMine, and XCMS have made the routine analysis of dysregulated peaks feasible. LC-HRMS now allows high-throughput untargeted metabolomic profiling[39].

Untargeted metabolomics is employed with no previous assumptions aiming to define the whole changes in metabolome, either in cell, tissue, or whole organism [40]. Both targeted and untargeted metabolomics have their advantages and pitfalls. Targeted metabolomics focuses on the analysis of specific categories of metabolites with more selectivity and sensitivity. Meanwhile, untargeted metabolomics gives the chance to discover novel discoveries [41], as the coverage of the metabolome is only restricted by the methods of the sample preparation and the inherent sensitivity and specificity of the analytical technique employed [42]. Untargeted metabolomics analyze all detectable metabolites from the sample, including unknown compounds [43]. Untargeted metabolomics however is truly intended for discovery and is not limited to a predetermined list of metabolites or class of compounds, with the aim to span the breadth of the metabolome [44].

Untargeted metabolomics can be a suitable choice for NPS analysis as it is not tied to the chemical structure of the compounds [45]. As cannabis (NPS) remains the most widely used controlled drug across Europe and the cannabis market has evolved, with more highly concentrated products becoming increasingly obtainable. Untargeted MS metabolomics have been employed across other NPS classes as a detection tool, Bijlsma et al identified two novel key markers, scopoletin and N,N-bis(2-hydroxyethyl) dodecylamine, which could effectively differentiate between tobacco and herb-tobacco mixtures based on the ratio of their peak areas utilizing high-resolution MS [46]. Nitazenes ingestion can be confirmed by their metabolites and can be referred to as toxicometabolomics [47]. The nitazenes metabolic profile primarily examined etonitazene, clonitazene and isotonitazene. These substances were observed to undergo de-alkylation, resulting in the formation of primary urinary metabolites such as N-desalkyl and O-desalkyl species [10]. The metabolite can exhibit higher potency than parent drug, such N-des-ethyl etonitazene, it exhibits agonistic activity to the μ-opioid receptors, identical to the parent compound [48]. However, metabolomics workflow can be applicable to detect exogenous source as within drug Phase I and Phase II metabolites [37, 47].

In summary, scientists made a success detecting metabolites of nitazenes either quantitatively or qualitatively. Metabolomics tools have been only applied in detecting and quantification of the metabolites of isotonitazene by Krotulski et al [10], which was among the earliest publications in detection and quantification of isotonitazene and its metabolites. As it has been achieved along in other NPS, metabolomics can help understand the metabolic pathways of drugs. Phase I and phase II metabolism of the NPS benzodiazepine cloniprazepam have been studied in vitro using MS based metabolomics and metabolites of phase I have been identified after oxidation, reduction and hydroxylation of the nitro-group, phase II metabolites have been identified after glucuronidation using untargeted screening approach by the metabolomics software MZmine [49]. Findings of phase I nitazenes metabolites have been estimated but phase II metabolites may still be insignificant within nitazenes [50].

The characteristics of many nitazenes are not fully understood and consequently concerns of their potency are arising, as for example etonitazene is 1000 times more potent than morphine [51].

Metabolomics have been employed extensively for other drugs to extend the window of detection as researchers have been interested to employ metabolomics aiming to establish a wider window of detection for Gamma-hydroxybutyrate (GHB) [52-54]. The assessment of nitazenes toxicity using metabolomics is very limited [55]. Also, it has been stated that nitazenes are sold in plenty of forms, nasal spray, powders, intravenous and even can be vaped [56], so impact of each route on human metabolome is not known. Additionally, the analysis of nitazenes is complicated by several factors, including their stability issues, metabolism, further complicates detection in biological specimens, as unchanged drugs may not be present. The lack of reference standards related to their continued development adds another difficulty in quantification and validation. Publications in the area of nitazenes metabolomics remain very limited. Metabolomics may estimate the long- term addiction of nitazenes as the discovery of long-term heroin addiction biomarkers [17] and could also be useful for studies on nitazenes. With the increased emergence of NPS, especially the evolving nitazenes, highly advanced analytical tools should be considered as they can be very complicated novel drugs in forensic casework. Concerns for nitazenes arise with the expectation of being ingested in micro doses relative to their high potencies. However, findings from studies employing metabolomics tools with other NPS may provide insights into how these tools could be used for nitazenes. The recent advancements in metabolomics, particularly untargeted metabolomics, offer a promising approach to overcome these challenges. By enabling the comprehensive analysis of metabolites without prior knowledge, untargeted metabolomics can identify novel biomarkers and metabolic pathways, which could enhance detection capabilities and potentially extend the detection window for these substances.

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Combining Genetic Analysis with Forensic Toxicology to Distinguish Between Accidental and Intentional Overdoses

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In forensic toxicology, differentiating between unintentional and deliberate overdoses is a crucial problem. Conventional toxicological tests concentrate on identifying drugs but they do not reveal genetic predispositions that affect drug metabolism, addiction, or suicidal thoughts. To improve overdose determination, this article examines how forensic investigations can incorporate pharmacogenomics and next-generation sequencing (NGS). Genetic factors influencing drug metabolism, genetic susceptibilities to addiction and suicidal behaviour, epigenetic changes associated with mental health and NGS applications in toxicological pathway analysis are important subjects. The accuracy of forensic results can be increased by forensic scientists using these biological insights to determine whether an overdose was caused by intentional drug use or an unanticipated reaction.

Drug overdoses are an increasing public health and forensic concern, often classified as either intentional or accidental, depending on the circumstances surrounding the event. Accidental overdoses are typically the result of unintentional misuse (the wrong drug was prescribed, or an extra dose of a given drug was taken)[1], 'polydrug use' which is the use of more than one type of drug at the same time or immediately after each other [2], or unknown allergies to certain drugs administered during medical or surgical procedures. Intentional overdoses are often associated with self-harm, suicide attempts or even homicides. Distinguishing between these categories is critical in forensic investigations to determine legal proceedings. Typically, accidental overdoses involves multiple interacting drugs, with concentrations falling within the toxic range. In most cases of intentional overdoses, only a single drug is used, or a known lethal mixture, but it is found in concentrations often many times higher than the lethal threshold[3]. NGS is an up-and-coming technique that has the ability to sequence millions of DNA molecules simultaneously. While traditionally used for DNA sequencing and profile generation, it can also be used to provide insights into toxicology and aid in determining if an overdose was the result of an unfortunate mistake or a deliberate act[4].

Workflow for Determining Accidental and intentional Overdoses:

The typical step-by-step procedure for distinguishing between accidental and intentional overdoses can be seen overleaf:

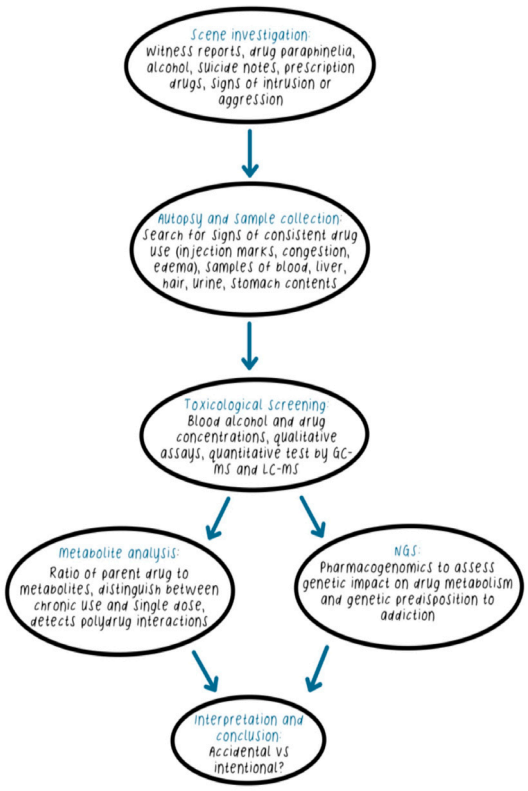


Figure 1 - Workflow for distinguishing between intentional and accidental deaths by drug overdose (authors own image)

Challenges in Identifying Accidental and Intentional Overdoses:

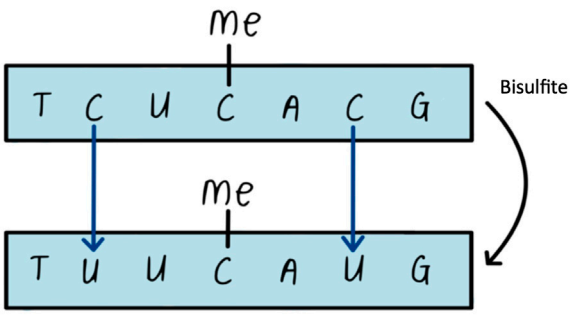
Identifying accidental and intentional overdoses can prove exceedingly difficult. A major challenge is the lack of clear toxicological cut-offs, as drug concentrations often overlap between therapeutic, toxic and lethal levels, as each individual will have varying tolerance. In cases of polydrug overdose, it can be difficult to pinpoint the exact cause of deaths. Postmortem redistribution also poses an issue as significant alterations in the blood drug concentrations can occur due to postmortem drug movement and instability, leading to inaccuracies[6].
Investigators must rely heavily on evidence present at the scene, such as drug paraphernalia, suicide notes, prescription drug history, but some cases provide little to no external context. To combat this, NGS can be used to help establish whether genetics played a role in the fatal overdose.

Table 1 - Comparison of accidental and intentional overdose indicators[1,3]

Factor	Accidental Overdose	Intentional Overdose
Drug Concentration	Within therapeutic or toxic ranges	Often at lethal levels
Polydrug Use	Common (usually alcohol and drugs)	May involve planned combinations (known lethal mixture)
Paraphernalia	Present	Usually absent
Scene Evidence	No suicide note, evidence of habitual use	Suicide note, methodical arrangement of drugs
Medical History	Substance abuse and past accidental overdoses	Depression, previous suicide attempts
Toxicological Findings	Drugs present at different metabolic stages	High amount of parent drugs, little metabolites

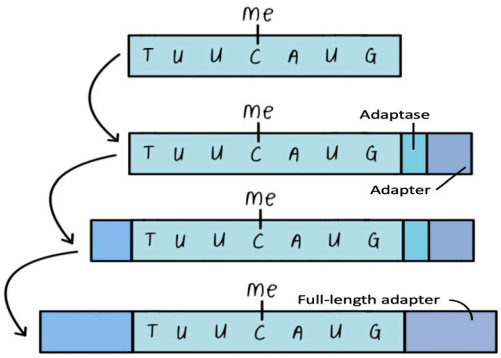
Overview of NGS:
NGS can generate masses of DNA sequences, as well as identifying transcriptional changes and genomic targets with base pair precision in response to chemical exposure[4]. It involves several major steps which include:
1. DNA fragmentation: this is done using high frequency sound waves or enzymes,
2. Library Preparation: Bisulfite conversion - fragments are treated with bisulfite, converting non-methylated cytosines into uracil's.

Figure 2 - Illustration of the bisulfite conversion process (authors own image)



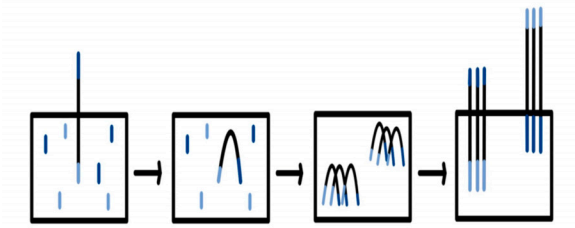
3. Library preparation: Methylation sequencing - allows the sequencing adaptors to be added to the DNA segments, which allows the sequencing primers to bind to all the DNA segments and enables massive parallel sequencing later.

Figure 3 - Illustration of the methalaytion sequencing process (authors own image)



4. Cluster formation – fragments hybridise and form a bridge and a double strand, which is then repeated several times. The double strand is then denatured to form single strands of DNA, forming dense clusters of identical DNA.

Figure 4 - Illustration of the bridge amplification and cluster formation process, (authors own image)



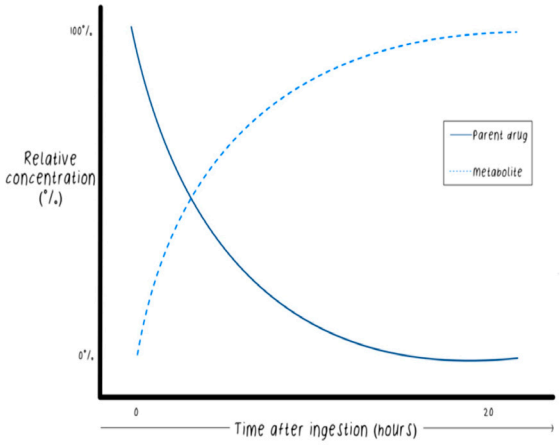
5. Sequencing – fluorescently-labelled flow chips are used which have tiny wells that capture a single DNA fragment. When each nucleotide is incorporated into the growing DNA strand, a phosphate ion is released, resulting in a flash of light. A camera captures the location and intensity of the light and a sequence can be created [7,8].

By integrating NGS with traditional toxicology, forensic experts can gain deeper insights into the biological factors that contribute to overdose cases, improving the accuracy of cause-of-death determinations.

Genetic Factors Affecting Drug Metabolism:
One of the main ways NGS can be adapted for toxicological analysis is by identifying genetic factors that influence an individual's risk of overdose. These genetic differences can affect how quickly or effectively the body absorbs, metabolises and eliminates drugs.

Once the drug has served its purpose, it needs to be deactivated and eliminated from the body. The function of drug metabolism is to convert lipophilic compounds (parent drug) into polar hydrophilic compounds (metabolites) to allow them to be easily dissolved in an aqueous environment such as blood, urine or bile and then excreted from the body [9].
Ratios of parent drug to metabolites can be examined to determine an approximate time of death. For example, heroin is a parent drug whose major metabolite is morphine. If high heroin levels are found, but low morphine levels, it suggests recent drug use and if low heroin levels and high morphine levels are seen, the drug was likely taken hours before death. Figure 5 below is a typical graph showing the decrease of parent drug and subsequent increase of metabolite concentrations over time.

Figure 5 - Graph showing the relative concentration of both parent drug and metabolite in % against time after ingestion in hours (authors own image)



Some commonly taken parent drugs and their metabolites are seen in table 2 overleaf:

Table 2 - Table 2 - Comparison of different parent drugs and their metabolites and their significance in forensic toxicology [10-16]

Parent Drug	Primary Metabolite (typically detected in blood or urine)	Significance in Forensics
Heroin	6-MAM; Morphine	Suggests heroin use (however, morphine alone is not exclusive to heroin as it can arise from codeine-based painkillers)
Cocaine	Benzoylecgonine; Cocaethylene (with alcohol)	Cocaethylene increases toxicity risk with alcohol
Cannabis	11-hydroxy-THC (active); THC-COOH	THC-COOH indicates it was taken weeks prior
Fentanyl	Norfentanyl	Detectable in urine for up to one week
Diazepam	Nordiazepam	Long half-life; helps in chronic vs. acute use determination
Paracetamol	NAPQI	Indicates severity of toxicity
Imipramine (Anti-depressant)	Desipramine	Parent-to-metabolite ratio used to determine type of overdose
Chlorpromazine	7-Hydroxychlor-promazine	Indicates potential psychotic episode

Pharmacogenomics is the study of how an individual's genetic makeup influences their response to drugs. This field combines pharmacology (the science of drugs) and genomics (the study of genes and their functions)[17].
One of the most well-characterised areas in pharmacogenetics involves the human cytochrome P450 (CYP) enzymes, which are primarily found in the liver. This family of enzymes is responsible for human drug metabolism and genetic variations in these may significantly alter how a person processes drugs [18].

CYP3A4 encodes an enzyme that is responsible for metabolising around 50% of all clinically used drugs. 19 CYP2D6 is responsible for the metabolism of opioids, such as codeine or morphine. CYP2C19 is responsible for metabolising many commonly prescribed medicines, including antidepressants, proton pump inhibitors and antiplatelet drugs [20].
NGS allows for the examination of these CYP enzyme gene variations that influence drug metabolism. Gene duplications make them ultra-rapid metabolisers, which could lead to extremely low levels of active drugs in the system but high metabolite levels, even at therapeutic doses. On the other hand, slow metabolisers may lead to an accumulation of toxic levels of drug due to impaired clearance [18].
This can aid investigators in estimating whether a drug was taken gradually and accumulated over time or all at once, resulting in sudden death by overdose.

Figure 6 - Graph comparing the relative concentration of parent drug and metabolite in % against time after ingestion in hours for both ultra-fast metabolisers and poor metabolisers
(authors own image)

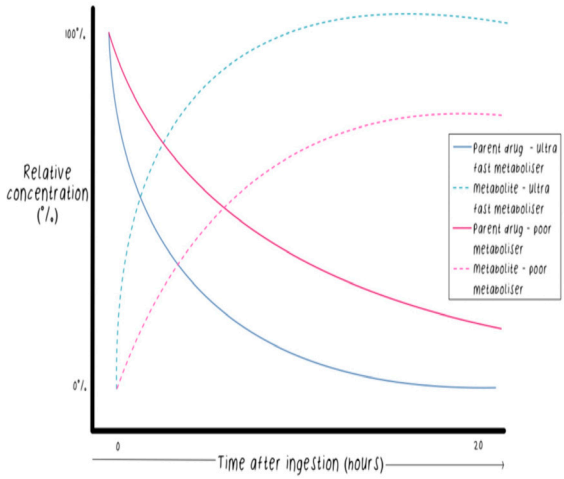


Figure 6 above shows a comparison of concentration of parent drug and metabolite against time after ingestion for both ultra-fast metabolisers and poor metabolisers. Ultra-metabolisers break down the parent drug rapidly, leading to low parent drug levels and high metabolite levels early on, while poor metabolisers process the drug much more slowly, resulting in higher parent drug concentrations and delayed metabolite formation.
By using NGS to identify genetic variants in drug- metabolising enzymes, forensic toxicologists can determine if a person's overdose was influenced by an underlying genetic predisposition to misprocess the drug. If genetic variants that suggest a heightened risk of impaired drug metabolism are revealed, accidental overdose is more likely.

NGS to Analyse Genetic Markers for Suicidal Behaviour and Addiction:
Familial research has shown that genetic variation significantly contributes to the occurrence of suicidal thoughts and behaviours, which includes suicide ideation, attempt and death [21].
The human serotonin transporter polymorphism (SERT) is encoded by a single gene, SLC6A4, with the most investigated region being the serotonin transporter gene promotor region, 5GTTLPR [22] brain-

derived neurotrophic factor gene, will lead to decreased BDNF suspected, which is linked to increased susceptibility to depression, anxiety and suicidal behaviours [24].

In forensic toxicology, NGS can be implemented in distinguishing intentional and accidental overdoses, especially in cases where the cause of death is ambiguous. While toxicology reports typically focus on drug concentrations and their potential lethality, genetic data can provide critical context by identifying an individual's predisposition to suicidal behaviour [25].

Addiction is a complex disorder influenced by both genetic and environmental factors, with studies estimating that genetic predisposition to addiction accounts for between 40 and 60% of an individual's vulnerability to substance abuse. 26 The SERT carrier has been associated with an increased risk for eating disorders, post-traumatic stress disorder and substance dependance [27]. The OPRM1 gene encodes the opioid receptor which is linked to increased susceptibility to opioid addiction[27]. Monoamine oxidase A is affiliated with compulsive drug-taking behaviour [28]. Variations of the dopamine receptor gene can lead to altered dopamine signalling, making some individuals more prone to drug-seeking behaviours and dependance [29].

If an individual carries genetic variant that will increase their vulnerability to substance abuse and has a known history of drug abuse, the overdose is more likely to be accidental caused by polydrug interactions or misjudged doses. However, if they carry variants that are associated with depression and suicidal tendencies, along with abnormally high doses, this may suggest intentional overdose.

NGS can also provide insight into epigenetic modifications, such as DNA methylation or histone acetylation, which can be influenced by chronic substance abuse and stress. Variants in genes that encode epigenetic modifiers can contribute to compulsive behaviour and heightened stress responders [30]. By analysing these epigenetic patterns, forensic toxicologists can assess whether an individual's substance use was driven by long-term addiction or acute emotional distress, further refining the distinction between accidental and intentional overdoses.

Conclusion

NGS offers a groundbreaking method for aiding in differentiating between unintentional and deliberate overdoses by fusing genetics and toxicology. Forensic scientists can create a thorough profile of a person's risk factors and pharmacological reactions by evaluating genetic characteristics that affect drug metabolism, vulnerabilities to addiction or suicidal thoughts, epigenetic changes, and toxicological pathways. Despite being a powerful tool for understanding genetic predispositions and drug responses, it should be viewed as a complementary method and not a sole determinant. Differentiating accidental and intentional overdoses requires a multi-factor approach, incorporating genetic analysis, standard toxicological analysis, case or patient history and scene evidence.

As technology and techniques become more advanced and more accessible, NGS has the potential to revolutionise forensic toxicology, particularly in overdose cases. However, its role should continue to be that of a complementary tool – one that enhances, rather than replaces established forensic practices in the task of distinguishing between accidental and intentional overdoses.

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Abbreviations

NGS – Next Generation Sequencing
CYP – Cytochrome P450
SERT – Serotonin Transporter
BDNF – Brain Derived Neurotrophic Factor

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CAREER SPOTLIGHT:

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Forensic Physician, Gold Coast University Hospital, Australia

Current Practice Areas/Industries
I'm currently working full-time as a forensic physician, which means I examine victims and perpetrators of interpersonal and sexual violence, write statements with expert opinion for the court in relation to injury interpretation, toxicology and at times, death investigation and I provide medical care to people who are in police custody.
I also have some other 'side-gigs' which include teaching GPs about recognising and responding to family violence, running workshops for other healthcare workers in recognising and responding to sexual violence and providing medical care to patients in a mental health ward at a private hospital.

Education
I completed my medical degree in 2010 at Monash University and in 2023 I completed a Master in Forensic Medicine. I now find myself studying a TAFE Diploma in Leadership and Management because the opportunity presented itself and I saw that there would be some value in it.
I completed my General Practitioner training in 2017 and was awarded FRACGP and am very close to finishing my specialist training with RCPA in Clinical Forensic Medicine (which will be awarded as FFCFM).

Did you always hope to work in toxicology? What did you do in school to prepare yourself for those opportunities?
Short answer – no. I think I fell into toxicology a few years after I fell into Clinical Forensic Medicine. If you asked me at age 16 what I wanted to be- it was a coroner. Although I realise now that actually I wanted to be a forensic pathologist and I just had the two roles confused. I wanted to do autopsies. But I got to medical school and realised I much preferred to work with the living, who usually smell better than the dead, so I started doing physician training thinking I'd do oncology. I loved the drugs in oncology; so complicated and with the most unusual side effects. That was probably my first clue that I'd end up loving toxicology.
Anyway, I quit physician training because I wanted to have a family and did General Practitioner training instead. As part of that, I found an opportunity to do a 6-month special skills elective in Clinical Forensic Medicine. And my 16-year-old self who used to watch documentaries on forensic science and aspired to be a doctor felt she had found her dream job. So I basically never left and ended up being part-time GP and part-time forensic physician for several years. But I always avoided toxicology because I thought it was too hard, until I decided to undertake my FFCFM training and then toxicology became unavoidable. And do you know... I loved it. It's a lot of time spent researching the literature and being very careful about how you express your opinions and I have had cases that have taken me a very long time to arrive at an answer that I am satisfied is accurate and defensible but I love the mental exercise of it.
What did I do in school to prepare? Well, I suppose the effort I put in to understanding chemistry has been actually very helpful now that I spend a lot of time looking at molecular structures of drugs and their isomers... I think as well even just starting on the path of wanting to be a forensic pathologist still somehow led me to where I am today. My advice would be to seek out and go for any opportunities that interest you. When I started GP training I never expected I would end up being a forensic physician who does toxicology but that is where following the opportunities took me and I have no regrets.

What work did you do following your initial undergraduate and postgraduate training if applicable? What further trainings did you undertake:
As I said, I originally started physician training and was studying for the first exam when I decided to pivot into General Practice. After I completed my Master of Forensic Medicine, I actually did an additional toxicology unit to understand the instruments used in analysis of specimens and interpretation of toxicology results better. I am still looking at further training opportunities but these need to be balanced out with everything else I do. I have been very fortunate to work with some very experienced forensic toxicologists who kindly share their expertise with me as I build up my own. I have also

recently joined The International Association of Forensic Toxicologists (TIAFT) and the Forensic and Clinical Toxicology Association (FACTA) and am starting to attend conferences and liaise with other clinical and forensic toxicologists.

What do/did you most enjoy about your work:
I love the variety of the work and the mix of patient-facing and non-patient-facing duties that I undertake. I enjoy the puzzle of it; what can I say, what can't I say, how do I qualify an opinion in such as way as to maintain its integrity and ability to stand up to cross-examination in court, have I been influenced by bias and how do I counter that?... putting together a robust statement of opinion for court even if it ends up being an opinion that doesn't land on one side or the other and is limited in the questions that it answers, takes a lot of care and consideration and an ability to see things from many different angles. I love the intellectual challenge. Toxicology in particular offers a lot of new challenges; especially when we get a weird or unusual opinion request.

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:
Probably nothing, to be honest. Even the years I spent in physician training still made me a better GP and forensic physician when the time came. The only regret I would have had is if I hadn't taken those opportunities as they came my way, for example the 6-month special skills placement in CFM, or the offer to stay on as a casual before becoming a permanent part-time forensic physician. Even moving from Victoria to Queensland, whilst difficult, has meant I have been able to do my CFM training with RCPA and hopefully very soon get my FFCFM fellowship.

What do you like to do in your free time:
Ha! I do not have free time. When I am not working my main focus is my sons. I focus on being present for them as much as I can and making sure I know what they're up to. Currently I work out at the gym and dance in the little spaces of time I can carve out for myself. Before I had children I did things like write and travel and I am sure I will do those again when the time is right.

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Drug Use and Misuse Trends in Older Adults Since 2010: A Review

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Keywords: Older Adults, Elderly, Drug Use, Drug Misuse, Drug Addiction, Drug, Psychoactive Substances

Introduction

A significant shift in societal views on illicit drug use occurred in the 1960s, resulting in increased drug use and misuse [1,2]. This has led researchers to identify the dangers and benefits of drug use, as well as manifestation of drug addiction disorders [3-5]. Those who were young adults during the 1960s are now considered older adults [6]. The World Health Organisation (WHO) defines "older adults" as those over the age of 60, whilst the UK National Health Service (NHS) considers only those over 65. The shift in recreational drug use perception impacted countries other than the UK and the USA later, slightly affecting the impacted generations. For the purposes of this study, "older adults" were defined as those over the age of 55. Currently, drug use and drug-related deaths are declining for every age group except older adults, for which both are increasing [7-9].

Additionally, the global population is ageing faster than ever [10]. Thus, toxicology research and toxicological interpretations need to consider the biochemistry of the older populations [11]. Older adults are known to have different drug metabolism, be prescribed more licit drugs (often simultaneously) and not manifest drug addiction symptoms similarly to their younger counterparts [11-13]. Furthermore, older adults are often excluded from drug use surveys and clinical trials [12]. Florisson and others [14] explain how the use of age limits and exclusion criteria that disproportionately exclude older adults must be critically evaluated to avoid the misrepresentation of older adults.

The above factors make the older population vulnerable to overprescription, unpredictable reactions to drugs (particularly in the case of polypharmacy) and under-diagnosing of drug addiction disorders [12,13,15-19]. As a result, the response to the drug-related inquiries disregards the upward trend in drug use and misuse in older adults.

This approach becomes inefficacious in an ageing society and results in misinterpretation of forensic and clinical toxicology casework [11,14].

The following review aims to cross examine the field of drug use research using information gathered from publicly available reports and discuss trends amongst older adults.

Method

For this study, data was collected from national health/statistics departments from the selected countries as outlined in Table 1 [7-9,20-23]. Because the definition of older adults is ambiguous, all age groups above 50 were considered [24,25]. To avoid misleading direct comparisons between countries due to population size, drug use/misuse was analysed as a percentage within each age group.

Data was collected from 2010 onwards to encompass the "Baby Boomer " generation (born between 1946 and 1964) most affected by the shift in societal views on drug use [26]. These trends were compared to research and case studies from hospitals, drug addiction recovery centres and independent researchers. Table 2 outlines the key words used for article selection. Only case studies in which the subjects were over the age of 55 were considered. All articles included were written in the English, Spanish or Portuguese languages.

Results and Discussion

Most analysed countries conduct yearly surveys on drug use trends and drug-related deaths and injuries. The European Union Drug Agency (EUDA) [20] developed a standardised data collection method but this is not adopted by every country, leading to issues with direct comparison of data. Additionally, its age range has an upper limit of 64, excluding older adults [20]. Similarly, England and Wales have a maximum age limit of 59 [7]. Scotland does not conduct a comprehensive drug use survey, but publishes an annual National Mission on Drugs report and collects data on drug-related deaths discriminated by more than five age groups between 25 to over 55 [8].

Countries such as the USA, New Zealand and Australia

Table 1: Selected countries and national drug report

Country	Report	Institution	Periodicity
Australia	National Drug Strategy Household Survey	Australian Institute of Health and Welfare	Tri-annual
Canada	Controlled and Illegal Drugs	Government of Canada	Annual
European Union	European Drug Report	EUDA	Annual
England and Wales	Drug misuse in England and Wales	Office of National Statistics	Annual
New Zealand	Annual update of key results: New Zealand Health	New Zealand Ministry of Health	Annual
Scotland	Drug-related deaths in Scotland	National Records of Scotland	Annual
USA	National Survey on Drug Use and Health	SAMHSA	Annual

Table 2: Relationship between keywords

Inital Topic	Similar Terms	Related Terms
Drug	Substances, Psychoactive substances	Cannabis, Cocaine, Opioids, Benzodiazepines, Opiates
Use	Consumption	Substance use
Addiction	Misuse, Abuse	Substance misuse, Substance abuse, Substance addiction
Older Adults	Elderly, Geriatric	Ageing, Pensioners

discriminate their population in more than five age groups with no maximum age limit [9,21,22]. Although the Canadian survey does not have an upper age limit, it only discriminates the population in two groups above or under the age of 25 [23]. This approach fails to identify trends in older adults when compared to younger adults. The older population is the largest consumer of benzodiazepines and opioids as prescription medication often simultaneously [30-34]. Despite being legal, these drugs can still be misused due to overprescription or self-medication, particularly when used long-term as they are addictive [30,34]. Benzodiazepines are a large group of drugs that include more than medications such as diazepam and alprazolam (Xanax) but also illicit substances such as etizolam [35,36]. In the 1960s the simultaneous use of multiple medications can cause heightened side effects which increase the chance of drug-related deaths and injuries [30-34,37-39]. Although these drugs are not mentioned in every examined report, there is a stark increase in benzodiazepine and opioid-related deaths in this age group in every report that mentions them [8,40-42]. Additionally, the age of patients entering heroin addiction treatment centres is increasing as more older adults seek treatment [20].

EUDA and WHO report that cannabis is the most used illicit drug worldwide [43,44]. Its use is increasing in the older population, particularly in countries where its recreational use has been legalised and as medication for chronic and/or psychiatric diseases [45-47]. Particularly in New Zealand, the reported recent use of cannabis has increased for all age groups above 55 as outlined in Figure 2. Nonetheless, in all countries examined there was an increase in cannabis use in the older population [7,9,20-23].

This review followed the guidelines proposed by Gregorich [48] for the literature review. However, the data analysed was sourced only from governmental reports on drug use/misuse. As discussed, the data collected for these reports lack in age discrimination, failing to emphasise trends in older adults. Currently, Copeland and others [49] are developing the National Programme on Substance Use Mortality (NPSUM) collating drug-related death reports from coroners across the UK (excluding Scotland). Although more thorough in their data collection than the Office of National Statistics, this data fails to include data on drug use that is not associated with drug-related deaths, such as cannabis. EUDA is carrying out an international project that aims to provide data on drug use habits across European countries through the combination of hair analysis with self-reported surveying [50]. Although the combination of hair analysis and surveying can provide more accurate data on drug use habits, the collection of samples is carried out at music festivals, targeting mostly young adults who attend these events [50].

Figure 1: Number of cocaine-related deaths by age in Scotland over the age of 55 between 2010 and 2022 as reported by the National Records of Scotland

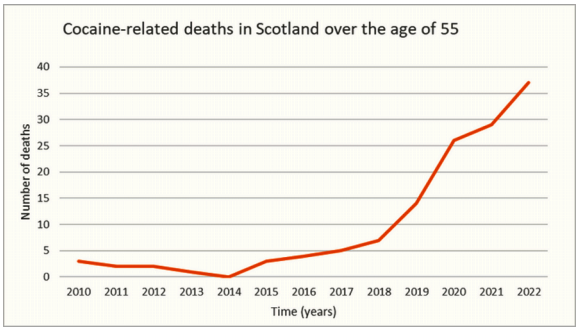
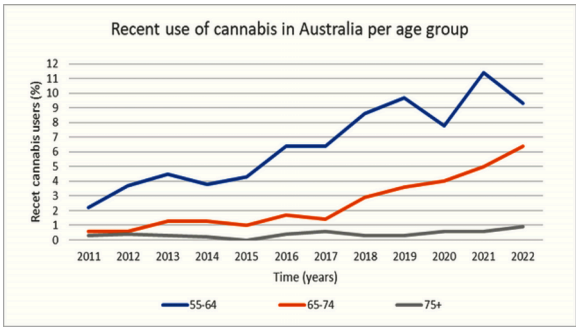


Figure 2: Percentage of reported recent use of cannabis in older adults in Australia per age group between 2010 and 2022 as reported by the Australian Institute of Health and Welfare



Conclusion

Ultimately, there is a clear upward trend on drug use and misuse in the older population which also reflect on increasing numbers of drug-related deaths. However, this demographic is neglected from drug research, clinical trials and drug use surveys. It is critical that the field of clinical toxicology adapts to the needs of an older population, to improve drug prescription, diagnosis of addiction disorders and addiction recovery approaches. In the future, larger-scale drug surveys would improve representativeness of the data, ideally including more age groups with narrower age ranges. Projects such as NPSUM and hair drug testing surveys by EUDA are beneficial for the identification of drug use trends in the population but still have limitations which could hinder the representation of older adults. Additionally, drug trials targeting older adults are required to understand the effects drugs (and their combinations) have on this age group.

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Cell Viability Assays for Use in Determination of Toxicology

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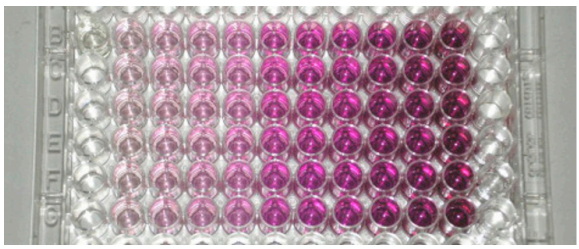
Keywords: Cell Viability, Dye Exclusion Test

Cell viability assays are an in vitro analysis of a compound which may determine a cytotoxic effect on a particular cell line. In this process, the cells are treated in media with the desired compound or material and growth of these cells are compared to that of untreated cells. The compounds can affect the cells in numerous ways causing toxicity such as enzymatic reactions, protein synthesis prevention and cell membrane destruction [1]. These have traditionally been used for drug development, anti-cancer treatments and medical devices but in recent years has been used to access the potential toxicity of compounds formed in water disinfection, development of compounds for animal feed and polymer degradation [2–7]. The assays can use a variety of different foundations to analyse the toxicological effects of using bioluminescent and fluorescent techniques, lactate dehydrogenase release (LDH), colorimetric dye test and the dye exclusion tests [8,9].

Fluorometric assays use fluorescence to examine the cytotoxic or cell proliferation effects on both suspended and adherent cell lines such as Alamar Blue, CFDA-AM and the GF-AFC assay.
In the case of Alamar Blue, a dye such as resazurin is converted from blue nonfluorescent to resorufin, a red highly fluorescent compound, when viable live cells are present [10]. Other assays can be utilised to utilised to examine more than just cell proliferation with bioluminescence assays being used to examine the effects of stress on the cell with them not requiring an external light source that used in fluorescent assays.

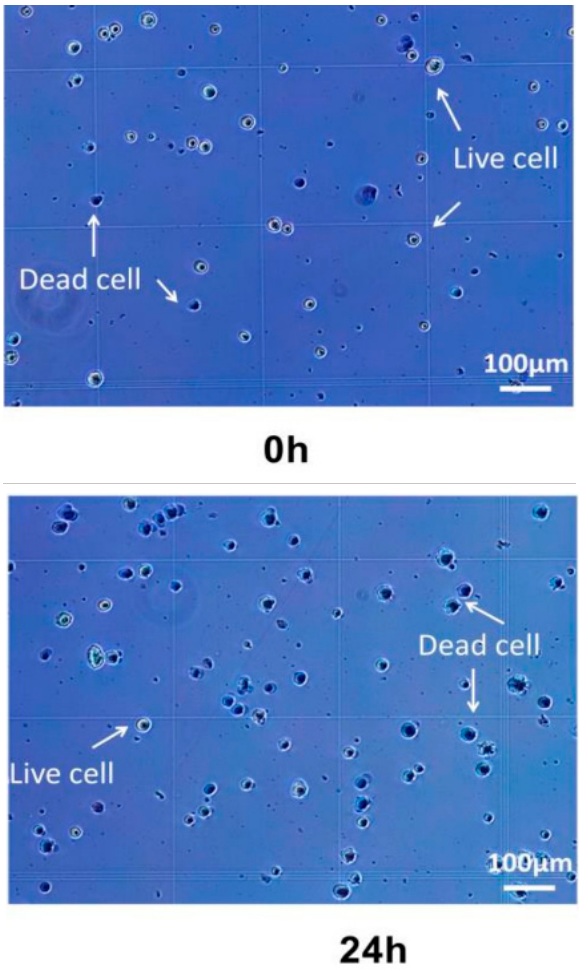
The luciferin-luciferase reaction is used to show when oxygen causes oxidation of D-luciferin, when magnesium ions and ATP are present using the catalyst firefly luciferase [11]. This is primarily used to examine the effects of external factors such as the introduction of drugs to the cell, with them examining the effects on the cell rather than the death of the cell. To examine the membrane damage of cells the LDH assays can be used, as lactate dehydrogenase is released from the cell membrane when it is damaged, the cell itself is damaged or due to cell death examining the quantity of LDH can identify cytotoxic effects on the cell [12].

Figure 1: MTT 96 Well Plate



Increasing concentrations of cells from left to right illustrating increase in purple formazan. Reproduced with permission [13]. Copyright (2017), Elsevier.
Colorimetric dye test such as the MTT assay use the conversion of a yellow dye MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to the purple coloured formazan [14](see Figure 1). Here a cell population that has not been treated is used as 100% and treated cells are compared to this to give a cell viability and is standardised in ISO 10993-5. 40% or below is strongly cytotoxic, 40-60% us moderately cytotoxic, 60-80% weakly cytotoxic and 80-100% is considered nontoxic [3]. A dye exclusion test such as Trypan Blue assay uses the same principles but here the living cell membrane prevents the dye from entering a cell and the only the dead cells take up the dye [5] (see Figure 2).

Figure 2: Comparison of Trypan Blue Assay Before and After Treatment
Reproduced with permission [16].



The cells line used in the assays can be dependent on the type of analysis being carried out with a variety of different cells lines being used. Cancerous cell lines such as MCF-7 breast cancer cells would be typically be used for accessing the anticancer properties of a pharmaceutical compound but the cancerous Caco-2 cell line (a colon cancer cell line) is used to assess compounds that can be ingested to see if they are toxic [17,18]. Non-cancerous cell lines are also used in toxicology, endothelial cells that originate from the interior of blood vessels have been used to assess the toxicity of nanoparticles used in biomedical material [19].

The assays have a number of advantages:
1. These assays can provide a quick and easy analysis of an unknown compounds toxicology and can enable the screening of a large number of samples [20].
2. As previously described, there are a variety of different assays and cell lines that can be used for different applications making them very versatile.
3. The data obtained from the assays is measured in absorbance, fluorescence or luminescence and is quantifiable allowing for it to be statistically analysed.

And disadvantages:
1. Cell viability assay can require a considerable amount of previous experience to be capable of completing and can take considerable preparation time with large amounts of plastic waste.
2. The assays cannot be used for long term exposure and can only be used for periods of 1-7 days [21].
3. The assays only replicate a certain aspect of the body and do not represent it as a whole, as such they cannot show the effects of the compounds after they have been metabolised or the effects of the immune response of the body [22].

Cell viability assays could potentially be a useful tool in the assessment of toxicology especially with unknown substances. by replicating the same concentration of compound found in a body. This would quickly show if that concentration was toxic but the cell line and assay used would have to be specifically chosen for the application. The MTT assay could easily be standardized for this purpose as it has already been standardised (ISO 10993-5) for the evaluation of medical devices. This would provide a quick and easy method for toxicity analysis as long as there has been sufficient preparation and training.

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Science in Motion - Fields Beyond Toxicology

Person-Centred Practice in Action: A Case Study From Practice Placement Learning

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Keywords: Person-Centred Care, Person-Centredness, Disability
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Introduction

Person-centredness is a global movement in healthcare that emphasises the importance of recognising and valuing the unique human experience of everyone receiving health and social care. Rather than focusing solely on disease or clinical outcomes, person-centred care promotes partnership, respect, empathy and a shared decision-making process between the healthcare professionals and the people they support[1].

In nursing, this philosophy has deep historical roots, aligning with the profession's core values of compassion, respect, dignity and holistic care. However, the consistent implementation of person-centredness in everyday practice remains challenging. Factors such as task-driven routines, organisational pressures and hierarchical structures can undermine the relational aspects of care [2]. To embed person-centredness meaningfully, there must be a sustained commitment to developing healthful workplace cultures - environments that support reflective practice, collaborative leadership and staff well-being[3]. Such cultures empower nurses and other healthcare professionals to engage authentically with persons they support and with one another, ensuring that care is not only clinically effective but also personally meaningful.

From my personal experience as a third-year student nurse in clinical practice I will reflect on a recent case in which I applied a person-centred approach while supporting a young child, her mother and her family. The young child who I will refer to as Susie (pseudonym) is three years old and has a clinical diagnosis of type 3 Osteogenesis Imperfecta (OI) also known as brittle bone disease. Osteogenesis Imperfecta is acommon genetic condition that effects the production of collagen, which prevents the correct formation of bone structure throughout the whole body. This causes the bones to become weak and fragile allowing dislocations of joints and fractures which may occur very easily[4]. Susie has developmental delays and is unable to speak using vocalisations to express herself.

It was important that both the child (Susie) and her mother were at the centre of all decision- making processes particularly when it came to meeting the needs of Susie. Trying to ensure that both Susie, her mother and family's choices were being facilitated was challenging and I will outline the impact this experience had on me, Susie and her mother.

It was important to support and acknowledge Susie's mother Rose (pseudonym) in this scenario as she cares for all her five children with a partner whom she claims does not offer any assistance. Rose has five children, including Susie under the age of 7 and her youngest child is a 6-month-old who was a twin. Tragically the other twin died at 2 weeks old. Rose explained that life is difficult and very busy because she has to feed and care for all her children, and although she loves Susie very much, she finds the amount of time and support Susie requires overwhelming, especially at mealtimes. This feeling is well recognised in the literature and findings suggest that mothers who have children with a disability share higher stress levels, worsening physical and mental health, and higher parenting requirements than mothers of children without disabilities [5]. In certain cultural contexts, families with and without a disabled child may differ significantly from one another.

These families comprise a higher chance of encountering socioeconomic hardship and are subjected to prejudice because of a disability [6]. They have to manage extraordinary and protracted caregiving responsibilities, complicated interactions with disability services and have to continue offering support throughout the child's life into adolescence, and adulthood [7] As Rose came from the travelling community and has a child with physical and cognitive disabilities, she falls into this 'at risk' category and she has reported that she is "finding caring for her child difficult".

The key action taken in supporting Rose was providing support, understanding and empathy for her social circumstances. Our aim was to educate her in relation to Susie's wellbeing by explaining the importance of nutrition, feeding and spending quality time with her. Throughout a child's life, nutrition and health is crucial to allow physical growth, survival and mental development [8].

We encouraged Rose to position Susie into her chair in a 90-degree angle and encourage Susie to interact at the table and socialize with her siblings which would also assist with the development of Susie's speech. We spent time with Rose demonstrating how to further care and support Susie, we showed her how to position Susie at a 90-degree angle, we discussed and demonstrated how to stimulate and encourage Susie to eat and enjoy a healthy food balance. We welcomed Rose to ask questions so that she could fully understand why she needed to give this specialised care to Susie. We also advised Rose that we were there to support her and that if she was unable to continue to give Susie the level of care and support that was required that we were obliged to refer her situation to the multidisciplinary team. This allows each member of the team to reflect and evaluate on the child's progress/deterioration and where concerns, suggestions and interventions can be outlined allowing an updated plan to be developed by the team to offer further supports to all concerned[9].

In Susie's case, the multidisciplinary team raised concerns regarding aspects of her home environment that appeared to be affecting her comfort, stimulation and mealtime routine. It was noted that Susie was not consistently fed at appropriate times, and there seemed to be limited opportunities for emotional interaction between her and her mother. The physiotherapist reported that some recommended developmental exercises did not appear to have been completed and Rose shared that she struggled to find the time to carry them out.

The occupational therapist also identified concerns during her assessments, noting that Susie experienced reduced social interaction and environmental stimulation. Additionally, Susie was not always supported in the advised seated position and her bedroom was often dark during daytime visits, with the blinds kept closed. These findings suggested that Susie may have been spending extended periods in her cot.

In light of these concerns, the team agreed that further supports were required. The meeting concluded with a decision to involve social workers to ensure that both Susie and Rose received appropriate protection, guidance and support.

This was a particularly challenging and emotionally sensitive situation for all involved. Rose, as both a mother and primary carer, faced significant pressures and had very limited family support. She had not accessed higher levels of education and reported difficulty understanding some of the care instructions provided for Susie. Rose was also experiencing ongoing psychological distress, as she continued to grieve the loss of a previous child. It was evident that she cared deeply for Susie; however, due to the combination of emotional strain, reduced social supports and practical challenges, she struggled to meet all of Susie's physical, emotional and developmental needs. Consequently, Susie's wellbeing and development were being compromised. To safeguard her rights and prevent further deterioration, timely intervention was essential.

Conclusion

This case highlights the complex clinical, ethical and moral dilemmas that healthcare professionals may encounter when safeguarding vulnerable children. Such decisions are often shaped by wider cultural, social and emotional factors within the family environment. It is therefore crucial that adequate services and supports are available for mothers of children with disabilities to help them provide safe and effective care in their home.

By implementing a person-centred approach, this case demonstrates the importance of recognising and responding to the needs of both Susie and her mother, Rose. As a student nurse, this experience has enhanced my understanding of the importance of holistic assessment, multidisciplinary teamwork and compassionate safeguarding practice to support the wellbeing of both the child and their caregiver.

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Lidocaine Toxicity Following Tumescent Liposuction

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Keywords: Lignocaine, Lidocaine Toxicity, Tumescent Anesthesia, Liposuction, Monoethylglycinexylidide, Glycinexylidide

Tumescent anesthesia is a form of local anesthesia achieved by infiltrating diluted lidocaine (lignocaine) with adrenaline into the subcutaneous fat to create firm, swollen or puffed tissue. It has been increasingly used in liposuction (tumescent liposuction), with a recommended lidocaine dose of up to 35 mg/kg and later increased to 55 mg/kg[1,2]. This technique has become popular due to its non-invasive nature and can be performed outside hospitals, including in outpatient clinics. However, there are limited case reports of serious adverse events and deaths associated with the tumescent technique. Deaths described in the literature indicated the contributing causes as fluid overload and pulmonary thromboembolism following the procedures [3,4].

This report outlines a patient who developed lidocaine toxicity with a good recovery following tumescent liposuction with confirmed blood lidocaine and its metabolites levels. The patient has provided consent for this case report.

Case report

A 35-year-old female with no significant medical history underwent tumescent liposuction at an outpatient clinic. Liposuction was performed on the patient's neck, back and abdomen by an experienced plastic surgeon with an anesthetist present. The procedure lasted for four hours with a total of 4 L of 0.9% sodium chloride containing 35 mg/kg of lidocaine with adrenaline infiltrated subdermally and subcutaneously at the liposuction sites. Intravenous (IV) midazolam 20 mg, fentanyl 400 µg and propofol 100 mg were administered for sedation. The operation was uneventful.

In the recovery area, the patient became drowsy and confused with bilateral jerking movements suggestive of myoclonus. She reported a metallic taste, blurred vision, nausea and had a single episode of vomiting. Naloxone 100 µg IV was administered in two aliquots, followed by two aliquots of flumazenil 100 µg IV without clinical improvement. She was commenced on supplemental oxygen of 10 L/minute and treated with an IV lipid emulsion: a 100 mL bolus of Intralipid® 20% followed by 400 mL over 20 minutes. She was subsequently transferred to the local emergency department (ED) via ambulance. During transport, she was transiently hypotensive (blood pressure 81/40 mmHg), responding to 500 mL of Compound Sodium Lactate solution.

On arrival to ED, her vital signs were stable: heart rate 105 bpm, blood pressure 122/90 mmHg, respiratory rate 20 breaths/minute, oxygen saturation 95% on room air and temperature 36.5°C. She improved but was still confused. Pupils were 4mm and reactive. Cardiovascular, respiratory and neurological examinations were unremarkable. Dressings were noted over surgical wounds on the neck, back, abdomen and inner thighs, bilaterally.
Initial blood tests showed a mild leucocytosis (white cell count 15.0×10^9/L), mild anemia (hemoglobin 112 g/L), and hyponatremia (Na+ 133 mmol/L), with normal renal function. Liver function and potassium levels were not reported due to lipemia and a hemolyzed sample. A 12-lead ECG demonstrated sinus rhythm with T-wave inversions in leads III and V2–V4. There was no QRS widening (QRS duration 93 msec) and a normal QT interval (337 ms).

The patient was admitted for observation with resolution of confusion within four hours. She remained stable overnight and was discharged the following afternoon without complications. Blood sample taken approximately eight hours after the initiation of lidocaine infiltration and five hours after the procedure was completed, revealed lidocaine concentration of 1.7mg/L, and its metabolites – monoethylglycinexylidide (MEGX) 0.67 mg/L and glycinexylidide (GX) 0.19 mg/L as analysed by Liquid Chromatography-Mass Spectrometry.

Clinical manifestations of lidocaine toxicity range from mild to life-threatening conditions, including numbness of the tongue, lightheadedness, visual and auditory disturbances, muscular twitching, alteration of consciousness, convulsions and respiratory and/or cardiac arrest. Lidocaine toxicity was considered the diagnosis in this case due to the constellation of her clinical manifestations and the temporal relationship between the symptoms and lidocaine infiltration. There is a good relationship between signs and symptoms of lidocaine toxicity and blood lidocaine concentrations. Muscular twitching, and alteration of consciousness are associated with a blood lidocaine concentration of approximately 8 mg/L[5]. Lidocaine has a half-life of 0.7 to 1.8 hours[6]. Whilst the patient's blood concentration 5 hours after the event was only 1.7 mg/L, it is conceivable that the concentration was within a toxic range at the time of her symptoms. Lipid emulsion was used by onsite clinicians to treat toxicity from lidocaine in this case; however, the mechanism and efficacy were unclear. Flumazenil and naloxone were given on-site, aiming to reverse the effects of midazolam and fentanyl respectively, but there was no clinical response.

Despite the dose applied being within the recommended dose ranges, there being no observed trauma and the surgery being performed by a highly experienced surgeon who was assisted by an anesthetist, there are other potential factors that contributed to the lidocaine toxicity in this case. A transient surge of lidocaine absorbed into the systemic circulation might have played a role in her sudden deterioration. With excellent onsite care, prompt hospital transfer and ongoing supportive hospital care, a positive outcome was achieved. Following the patient's discharge home, the treating medical toxicologist had a meeting with the surgeon and the anesthetist to exchange experiences and to further collaborate with a pathway to consult medical toxicologists through the poison centre should a future event occur.

Lidocaine and its metabolites are not available for routine hospital testing. The levels were obtained in this case, which were subsequently helpful for diagnosis and case discussions. This case report highlights the importance of early diagnosis of lidocaine toxicity, appropriate onsite management and prompt referral to an emergency department for a higher level of care and ongoing monitoring in the hospital.

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Potential of Aptamers in Analytical Toxicology

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Keywords: Aptamers, Enantiomers

Abstract

Analytical toxicology plays a crucial role in the identification and quantification of xenobiotics, drugs and their metabolites in forensic science, clinical diagnostics, environmental forensics and other specialisations which test complex and often degraded or unstable matrices that require extensive sample preparation. Aptamer-based detection systems present a promising alternative to traditional detection methods with competitive sensitivity, specificity and selectivity, as well as cost and time efficiency.

Aptamers are short, single-stranded DNA or RNA sequences capable of selectively binding analytes to induce conformational changes and signal transduction making amenable to various detection methods. While the use of aptamers in clinical diagnostics and environmental has gained popularity, their application in analytical toxicology remains underexplored.

This review explores the potential of aptamers in analytical toxicology, describing their structure and mechanism and highlighting their advantages, challenges, current applications, and prospects with special emphasis on new psychoactive substances.

1. Introduction

Analytical Toxicology & Detection Methods

Analytical toxicology plays an important role in the detection, identification and quantification of drugs, xenobiotics (foreign compounds), other compounds and their metabolites in forensic science, environmental forensics, clinical diagnostics and food safety by detecting exogenous and endogenous compounds whose effects may change from beneficial to harmful depending on their concentration[1].

Analytes may be found in complex, and often degraded or unstable matrices which may require sample preparation and extraction techniques before analysis to remove interfering compounds or pre-concentrate the analyte to ensure its detectability[1]. While sample preparation techniques have evolved to reduce costs and improve time-effectiveness, versatility and simplicity, scientists "in field" often do not have the luxury of additional procedures, for various reasons such as time constraints, limited space and storage, transport and monetary limitations.

The development and use of aptamers and nanotechnology may counteract these restrictions, as well as improve sensitivity, specificity, selectivity and detection limits with minimal sample preparation and interference effects[2]. While aptamer technology is not new and nanotechnology is constantly advancing, its use in analytical toxicology and forensic science is limited, despite its many advantages compared to instrumental techniques and immunological assays. These include cost and time efficiency, stability and adaptability to a larger variety of molecules which will be discussed further in this article.

Aptamers

Aptamers are short sequences of single-stranded DNA (ss-DNA) or RNA, usually between 20 and 100 base pairs, used as biological sensors (biosensors) of drugs and biological target molecules such as proteins and bacteria [3,4]. Aptamers are bound to detectors and can selectively bind to target compounds, which result in their conformational change and signal transduction [4]. Aptamers incorporate versatile and extremely sensitive detectors, including colorimetric, electrochemical and fluorescent detection, making them usable with minimal training required.

The development of aptamers largely focused on their applications in clinical settings, particularly targeted therapy and diagnostics, and environmental forensics and food safety for detection of pharmaceutical products, illicit drugs and biological target molecules such as antibodies, hormones, and bacteria, but have under-used potential in analytical toxicology [4-7]. Aptamers may be advantageous in this field due to their sensitive and selective detection capability even in complex matrices, such as urine and saliva, with minimal to no interference and without the need for meticulous, complex, and oftentimes costly sample preparation [8].

2. Aptamers in Analytical Toxicology

2.1 Overview

Definition & Structure

As mentioned previously, aptamers are oligonucleotides, composed of 20-100 base pairs, that act as high-affinity ligands to detect a range of target molecules. The molecular weight of aptamers may vary from 10-20 kDa [9]. Aptamers' capabilities to detect, amplify and quantify signals generated by their interaction with target molecules in a compact device increase sensitivity and simplicity of an assay, and often improve production cost and market availability [2].

History

The development of aptamers originated in 1990, when Ellington and Szostak generated a library of "random-mers", random sequences of DNA with defined regions at the 5' and 3' ends needed for primer hybridization and cloning, with an average length of 100 bases [3]. These random sequences were then amplified into RNA strands by RNA Polymerase during Polymerase Chain Reaction (PCR), which were then able to bind specifically to small ligands [3].

Since then, many more aptamers have been developed using an iterative selection process called SELEX, performed *in vitro,* making the development process more efficient, less time-consuming and costly and helped aptamers become widely available for therapeutics, drug testing, environmental monitoring and other applications [5,10].

Development Process

The nucleotide sequences within aptamers are evaluated during the Systemic Evolution of Ligands by Exponential enrichment (SELEX) process, in which a large database of oligonucleotide sequences with large sequential and structural diversity (E13-E15 sequences) is screened for the components' binding affinities to an analyte [5,6,10].

During this process, the chosen analyte is immobilized onto a surface, such as beads, columns or chips and exposed to oligonucleotides to allow for the binding between the aptamer and target molecule [5,6]. The target-bound oligonucleotides are then isolated by removing the unbound sequences in a washing step, before being amplified and purified for further optimisation which may take 5-20 cycles [5,6,9].

Only oligonucleotides which can bind very tightly to the target molecule will continue to the subsequent selection rounds and the highest affinity sequences are chosen [5]. The selection of a particular sequence may depend on its separation efficiency, discrimination power between structurally similar analytes, stability and potential binding possibilities by modification of functional groups [5]. As a result, the SELEX process acts as a validation process of several oligonucleotides for a specific target ensuring high selectivity.

Advantages

Aptamers have proven to be advantageous to chemical detectors, instrumental techniques and immunological assays, with their improved sensitivity, precision, resistance to interference effects, development time and cost. Aptamers have achieved detection limits in the nano- to pico-molar ranges [6,11].

Both chemical detectors and instrumental methods may need extensive sample preparation, may be large and cumbersome, making them difficult to transport and use "in the field". Furthermore, they require regular maintenance, calibration and validation, which may be time and financially costly and need trained operators. While recent sample preparation methods, such as QuEChERS and Solid-Phase or Liquid-Phase Micro-Extraction (SPME and LPME, respectively) have revolutionised preparation procedures, knowledge and careful consideration of the correct preparation method is still necessary which may impede time-critical investigations. Furthermore, optimisation of sample preparation techniques for a particular matrix and analyte may take months or years to complete, again a luxury which oftentimes is absent from casework.

On the other hand, while immunological assays may need smaller and cheaper instrumentation to perform than analytical techniques, it may take prolonged periods of time to develop and validate an assay. For example, it takes a comparably much shorter time to develop aptamers (days to weeks) than antibodies (weeks)[9]. Furthermore, immunoassays are costly due to the production costs of antibodies in animal models, which must also be extensively validated with only highly immunogenic compounds to ensure high affinity and a detectable response [6,11].

Aptamers may provide a greater analyte range, such as metal ions and poorly immunogenic or highly toxic molecules which are not adaptable to immunoassays[11].

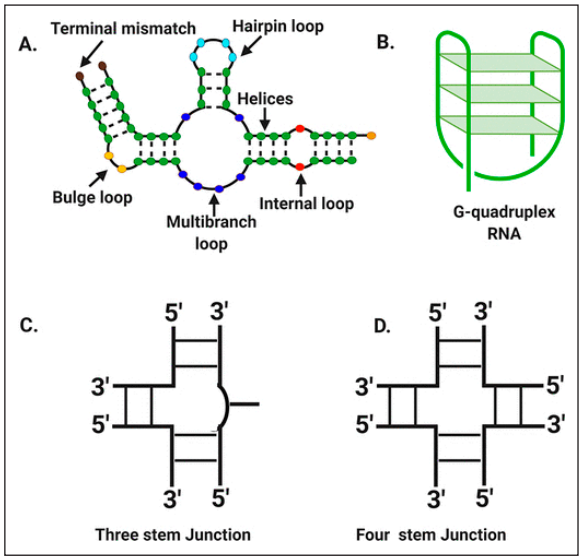
Additionally, aptamers are easier to obtain, less prone to biodegradation and denaturation at a range of ionic conditions than antibodies [6,9-11]. Aptamers may become preferred due to their reduced batch-to-batch variability and cost reduced by several orders of magnitude [9].

2.2 Mechanism

Conformational Changes

Aptamers undergo conformational changes in the presence of their analyte that can be detected when functionalised to nanoparticles or sensor surfaces [10]. The binding of an aptamer to its analyte results in hairpin loops, bulge loops, multibranch loops, internal loops, junctions and G-quadruplex, seen in Figure 1 [12]. Several forces such as hydrogen-bonding, pi-pi stacking, van der Waals forces and hydrophobic effects contribute to the specific three-dimensional structures of aptamers [13,14].

Figure 1: Schematic Diagram of Aptamer Structures



(A) Different stem and loop structures, forming hairpin loop, bulge loop, multibranch loop, internal loop and helices. (B) G-quadruplex. (C) Three stem junction and (D) Four stem junction [12].

Binding Affinity

The binding affinity between an aptamer and a target molecule is compared by K^d values, which is the dissociation constant that characterises the optimum concentration of an aptamer to bind to a particular target [13,14]. A lower K^d value indicates a stronger binding affinity between the aptamer and target molecule, which helps scientists identify promising aptamers for a chosen target.

Detection Methods

Aptamers are amenable to a range of detection methods as they are immobilised onto a surface, which may be a colorimetric, fluorescent or electrochemical detector [15,16]. Colorimetric aptasensors tend to exploit gold nanoparticle (NP) or other nanotechnology, while fluorescent methods exploit intercalating agents that bind between the G-quadruplex structures within the DNA sequence [10,16,17]. Aptasensors have been developed for a range of analytes with various detection methods, summarised in Table 1 [4,8,10,15,17-28].

Table 1: Summary of Analytes and Their Corresponding Aptamer-Based Detection Methods [4,8,10,15,17-28]

Analyte	Method		
	Colorimetric	Fluorescent	Electrochemical
Cyanide	✓		✓
Heavy Metals (Pb, Ag, As, Cd, Hg)	✓	✓	✓
Antibiotics	✓	✓	✓
Cocaine, methamphetamines and amphetamines	✓	✓	✓
Pesticides & Insecticides	✓		
Pharmaceutical & veterinary drugs	✓	✓	✓
Hormone disruptors (BPA, 17β-estradiol, DES)		✓	✓

2.3 Applications

Separation of Target Molecules and Enantiomers

It is widely known that enantiomers of biomolecules and pharmaceuticals may result in unwanted and sometimes even dangerous side effects, giving rise to the need for separation of enantiomers during the manufacturing process. Furthermore, in certain circumstances where legislation bans one enantiomer but not the other, it may be beneficial to separate structurally similar compounds such as Δ^8-THC and Δ^9-THC.

Aptamers have been used as immobilised ligands to separate non-target molecules and enantiomers of small bioactive molecules and proteins, such as adenosine, tyrosinamide and thrombin, and pharmaceuticals in capillary electrophoresis (CE) and chromatography [29,30].

Detection of Illicit Drugs

The quantification of regulated drugs is one of the areas within analytical toxicology, due to their inherent toxicity and high abuse potential. Aptamers for the detection of several illicit drugs have been developed, largely for cocaine, methamphetamines and amphetamines with novel aptamers being developed for fentanyl derivatives [24].

Colorimetric aptasensors have been reported for the detection of a series of opioids, including heroin, morphine, codeine, and hydrocodone in pharmaceutical tablets, drug-drug mixtures and drug-interferent mixtures [31]. These aptasensors distinguished between drugs with similar functional groups, giving accurate detection of multiple analytes in drug mixtures, and proved superior to the presumptive Marquis test when used as a comparison [31]. The authors achieved detection limits (LOD) of 0.5 µM [31]. Another colorimetric aptasensor for cocaine and adenosine was developed using gold NPs with a LOD of 0.3 mM in aqueous solution [10].

Fluorescent aptasensors have been developed for methamphetamine and cocaine, with detection limits of 0.78 nM and 50 nM, respectively [28,32]. Increased development of aptasensors could lead to the addition of specific aptamers for date-rape drugs to the established collection.

Detection of Pollutants in Water

There is a variety of pollutants, ranging from pharmaceuticals such as antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs), which are commonly found in households, to potent hospital-use drugs, such as cancer treatment, anaesthetics and hormones [4]. The presence of these residues in surface, ground and drinking water often arises from human and animal treatment but can have a lasting impact both on human and environmental health [5].

The contamination of water by antibiotics, cancer-treating drugs, and hormones can contribute to the evolution of antibiotic-resistant microbes and the increased prevalence of cancer in aquatic life, which may also directly impact human health through the consumption of contaminated seafood.

A wide range of aptasensors have been developed for pharmaceutical compounds which contaminate water bodies, which incorporate fluorometric, colorimetric and electrochemical detection [4].

Detection of Vitamins

While vitamins are necessary trace components of a balanced diet which play vital roles in many physiological systems, too much of something good may be harmful, a common concept in analytical toxicology. Although water-soluble vitamins can be relatively easily eliminated from the body through urine, antioxidants such as Vitamin C may remove other vital compounds. Furthermore, fat-soluble vitamins may accumulate in the body, particularly the liver, which may cause unintentional health problems.

Aptamers have been used to quantify several vitamins and supplements, such as riboflavin (Vitamin B2), nicotinamide (Vitamin B3), and biotin (Vitamin B7) [18,19,29].

As such, the detectability of vitamins by aptamers can be useful in cases of accidental overdoses or suspicious circumstances. Additionally, aptamers can be used in clinical toxicology to assess nutritional status and prevent comorbid diseases resulting from vitamin or mineral deficiencies.

Detection of Heavy Metals

The consumption of heavy metals, such as lead, thallium, arsenic and cadmium, can lead to severe health problems and even death after prolonged exposure as the metal ions accumulate in bones, hair, skin, and organs[25]. Heavy metals persist as environmental pollutants, but may also be found in poorly regulated pharmaceuticals, cosmetic products and female hygiene products. The acceptable blood levels for heavy metals are very low, being in the ppm and ppb ranges [25]. As a result, heavy metals are often quantified with ICP-MS, ICP-OES, and GFAAS, which have very high initial and operational costs and need skilled operators and technicians.

As an alternative detection method, using aptamers for the detection of metal ions can significantly improve cost and time efficiency, making it available to more laboratories. Already, several aptamers have been developed to detect and quantify heavy metals such as thallium (I), lead (II), arsenic, mercury (II), cadmium (II), and silver (I), with excellent detection limits, listed in Table 2 [16,25,33-34].

Aptamer structures involved in heavy metal detection are the G-quadruplex, hairpin loops, and stem loops [23,25].

Table 2: Aptamer-Based Methods for the Detection of Heavy Metals and their Limits of Detection (LOD) [16,17,23,33,34]

Metal	Detection Method	LOD	Surface	Indicator	Reference
Pb(II)	Fluorometric	0.84 nM	In Solution	Berberine	[16]
Tl(I)	Fluorometric	3.4 ! M	In Solution	Berberine	[16]
Pb(II)	Fluorometric	1.6 nM	Paper	Berberine	[16]
Tl(I)	Fluorometric	1.1 nM	Paper	Berberine	[16]
Pb(II)	Electrochemical	0.4 nM	Amperometer	None	[17]
Hg(II)	Colorimetric	0.6 nM	In Solution	Gold NP	[33]
Pb(II)	Electrochemical	10 pM	Impedometer	Ferrocyanide	[34]
Hg(II)	Electrochemical	0.1 nM	Impedometer	Ferrocyanide	[34]
Ag(I)	Electrochemical	10 nM	Impedometer	Ferrocyanide	[34]
Cd(II)	Colorimetric	4.6 nM	Polymer	Gold NP	[23]

3. Challenges & Limitations

Degradation by Nuclease

Aptamers, particularly RNA-based aptamers, are easily degraded by nuclease activity, unless modified [6,35]. However, this limitation has already been overcome by chemical modification that increased stability [35].

Amenability to Large Target Molecules

Large target molecules, such as hormones, antigens, peptides and other biomolecules, are more amenable to aptamer detection due to their larger structures and surface area available for binding [36]. On the other hand, small molecules, such as chemical compounds and metal ions, have smaller molecular weights and simpler structures than large biological target molecules such as proteins [36]. As a result, small molecules pose difficulty in binding to aptamers due to fewer binding sites [36].

The screening process of aptamers requires the immobilisation of the target molecule, which is difficult to perform on small molecules, making the development process for small-molecule-specific aptamers challenging. However, this can be overcome

by using capillary electrophoresis (CE) SELEX, which removes the need to immobilise the target molecule[36].

Furthermore, the limited amenability to small molecules can be overcome by artificially increasing the size of chemical targets by conjugating to a fluorescent or radioactive label, as has been done in the detection of cocaine and other illicit drugs.

4. Prospects & Emerging Technologies

Detection of Human DNA

Multiple DNA-based aptamers have been developed to simultaneously detect several types of Streptococcus pyogenes, a significant pathogenic species for humans, with very good K^d values, as low as 4 nM[37]. The authors highlight two aptamers with particularly promising dissociation constants of 9 nM and 10 nM[37]. The development of several aptamers with such high affinity for a bacterial strain, along with aptamers targeting other biological targets such as tissues, cancer cells and viruses, poses the possibility of the creation of a similar aptamer for the detection of human DNA for forensic use in crime scenes and contaminated food[6]. This new aptamer could reduce the costs or remove the necessity for lateral flow immunoassays such as the RSID™ kits (Galantos Genetics GmbH) that detect human saliva, semen, blood and urine or give scientists a more cost-effective option with similar specificity to antibodies[38].

The detection of saliva may prove useful not only for the collection of DNA, but also to prove or disprove statements made by injured parties or suspects, particularly in sexual assault related cases. Alternative light sources (ALS) have limited use for saliva detection due to low fluorescence exhibited by this sample. Presumptive tests for alpha-amylase, a component of saliva but also of other bodily fluids, such as the Phadebas™ test have been extensively used in forensic science, but this method has the limitation of poor time efficiency and detection limits being dependent on the levels of alpha-amylase in the saliva, which itself varies intra and inter-individually, depending on time since food consumption and genetic factors.

The detection of saliva by aptamers was described by Li *et al*. who developed an aptasensor for simultaneous detection of two oral bacterial species in saliva (*S. salivarius* and *S. sanguinis*) using red and blue fluorescence[39]. Furthermore, aptasensors for the human saliva-specific RNA markers, *STATH* and *HTN3*, could be developed as these biomarkers have been quantified with RT-PCR[40]. Using these biomarkers as targets in aptasensors could improve specificity compared to alpha-amylase-based detection techniques such as Phadebas press test, as alpha-amylase is present in other body fluids[40].

Dual Detection of Target Molecules

In therapeutics, aptamers can simultaneously bind covalent and non-covalent targets[6]. As a result, taking advantage of this property can result in the development of aptamers that are highly specific for two targets, for example, a metal ion and an illicit drug, which can further expand aptamer applications in analytical toxicology.

Separation of Psychoactive Enantiomers & Detection of Regulated Drugs

As aptamers have been used in the separation of enantiomers, this property of aptamers can be exploited by analytical toxicologists and law enforcement for monitoring of new psychoactive substances and derivatives of illicit drugs which have not been regulated or discovered. Aptamers may be used in the discovery of NPS, which have been theorised but not discovered in the illegal drug market.

The high selectivity of aptamers may be advantageous than other separation methods, which have struggled to separate enantiomers like THC-derivatives due to their extremely high similarity, which has required NMR for successful identification.

Additionally, aptasensors for the detection of highly potent illicit drugs such as fentanyl, fentanyl derivatives, nitazenes and other novel psychoactive substances (NPS) have been underexplored despite their considerable potential, especially in overdose cases and in customs monitoring. These NPS offer grave consequences even in minute doses, a particular concern for customs officers, laboratory analysts, law enforcement, and any others who may encounter these illicit drugs. Aptasensors may reduce the high risks associated with handling these substances due to their high sensitivity and specificity.

5. Conclusion

Undoubtedly, aptasensors already have many applications in analytical toxicology due to their competitive sensitivity, specificity, production cost, time and detection time. Further development of aptasensors in this speciality is needed and has many underexplored applications, especially in the separation of enantiomers of psychoactive drugs, which have become of particular concern in the emergence of new psychoactive substances (NPS). Aptasensors could reduce risks associated with handling potent drugs due to their low limits of detection, as well as help in the proactive in-field discovery of NPS, which have only been theorised to date.

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From Synthetic Ligands to Botanical Complexities: The Analytical and Clinical Evolution of the Australian Poisonous Plants Project

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The pivot from the high-stakes, rapid-turnaround environment of frontline drug checking (or "pill testing") to the systematic revitalization of the Australian Poisonous Plants Project (APPP) [1] - a legacy initiative dating back to 1984 - represents more than a simple shift in subject matter. For the analytical chemist and the senior medical practitioner alike, it represents a profound evolution in managing the interface between human physiology and environmental chemical threats. While the "pills" of the last two decades and the "plants" first catalogued in the 1980s may seem disparate, they are unified by the necessity of high-resolution analytical surveillance, the clinical application of toxidromic recognition and a public health requirement to involve end-users with relevant point-of-deployment messaging.

The Analytical Frontier: From POC to High-Resolution

Our work at festivals with Pill Testing Australia [2] and at our fixed site, CanTEST [3], was built on a foundation of point-of-care (POC) analytical chemistry. For the clinician, the challenge was the "unknown ingestion"; for the chemist, it was the "interference-rich matrix". In the festival environment, we rely heavily on Fourier-transform infrared (FTIR) spectroscopy. From a chemical perspective, FTIR provides an elegant, non-destructive method for identifying bulk constituents by measuring the absorption of infrared radiation as it excites molecular vibrations. We conduct these analyses in front of users - like a "close-quarters magic trick" - explaining every step to build trust with a demographic that understandably fears and anticipates 'judgment'. In addition to the medical and technical aspects of POC pill testing, we have spent considerable efforts over many years to ensure the most ethical experience possible [4].

However, the Australian illicit market has experienced a predictable "potency shift". We have moved quantitatively from MDMA (active at 75–100 mg) to higher purity levels and qualitatively toward synthetic opioids and substituted cathinones active at the microgram level. This rendered FTIR - with its roughly 5% limit of detection (LOD) - less useful as a definitive qualitative arbiter. Consequently, we deployed Ultra Performance Liquid Chromatography (UPLC) with Photodiode Array (PDA) detectors at our fixed site and more recently a mobile Liquid Chromatography-Mass Spectrometry (LC-UV-MS) unit, affectionately dubbed "Quasimodo" to our festival service.

To understand the current "re-germination" of the APPP, we must acknowledge its 1984 origins as a community-funded project, to delineate Australia's toxic flora for human safety [1]. Until that point, much of the effort in the field was for the benefit of pastoralists; it was a taxonomical masterpiece that remained largely isolated from human clinical medicine.

We have started by re-evaluating the literature, using electronic literature searches that are well-established now but were only just becoming available in the 1980s. Our team has searched the scientific and medical literature for any record of plants causing harm to humans but also the historical, sociological and ethnobotanical literature for any further records.

Most collections suggest there might be around 1000 plants practically toxic to humans using a methodical, painstaking search strategy, we have identified to date just under 1500, or around 5% of all Australian plants. Hundreds of these have yet to have the toxic principle identified.

The reader will appreciate the comparative ease with which a large language learning model might be trained on a set of plants already identified by experts as being toxic, rather than the global entirety of all plants in existence. This provides a model that might be deployed in other jurisdictions. The process is underway with the Computer Science Department at the ANU.

We have recently presented our first project -deaths from poisonous plants in Australia over the last 25 years -a collaboration with the National Coronial Inquest System (NCIS) at The International Association of Forensic Toxicology (TIAFT) meeting in Auckland New Zealand- you can hear about it here [5].

The next steps will be to start collating the rich tapestry of stories linked to poisonous plants in Australia and to encourage citizen scientists to contribute their narratives and pictures, acting as 'biosensors' themselves, to build a bigger richer picture of the continent.
The final phase will be the most complex, yet conceptually not dissimilar to the concepts deployed in pill testing analysis. The botanical world remains an unregulated laboratory of secondary metabolites - alkaloids, glycosides and terpenoids - evolved over eons to target the very receptors we manipulate in modern medicine. Unlike a recreational drug, containing two or three active ingredients, a specimen like Jimson Weed *(Datura stramonium)* is a multicomponent matrix. Chemists face the daunting task of identifying specific tropane alkaloids (Hyoscyamine, Scopolamine) against a background of thousands of plant proteins and lipids.
The APPP relaunch involves a significant evolution from that which we used in POC festival testing to Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and Orbitrap technology. This will allow us to identify highly potent molecules with sub-ppm mass accuracy and use Collision-Induced Dissociation (CID) to reconstruct the "molecular skeleton" of unknown alkaloids.

This has been critical elsewhere for species like the Gympie-Gympie *(Dendrocnide moroides)* the infamous Australian stinging tree. For decades "moroidin" has been unconvincingly identified as the toxic principle. Only in the last few years has modern High-Resolution Mass Spectrometry (HRMS) analysis of gympietides - complex miniproteins resembling those found in spider or cone snail venoms—reveals their ability to target voltage-gated sodium channels which may explain the refractory nature of the intense pain they cause [6].
We are adopting the "Sentinel" model used for drug checking and applying it to potential botanical crises which happen such as the 2022 tropane alkaloid spinach contamination. Should patients present with "red as a beet, dry as a bone" anticholinergic symptoms, the APPP might provide the translational link. By maintaining a database of "look-alike" species, we provide clinicians with real time data to inform clinical decisions. Linking environmental stressors like drought to increased alkaloid concentrations in "safe" crops.

A unique aspect of the modern APPP is the inclusion of consideration environmental stress factors such as drought or increased CO2, which alter plant metabolic pathways. Many species increase production of cyanogenic glycosides under stress. When hydrolyzed, these release Hydrogen Cyanide (HCN), leading to cellular hypoxia where cells cannot utilize oxygen due to the inhibition of Cytochrome Oxidases. These species are becoming more toxic. Similarly, Amanita spp are becoming more accustomed to their new living arrangements and are spreading, demonstrating the ability to switch hosts elsewhere [8]. Should that occur in Australia, the outdoors will become even more dangerous than it was before. The APPP will be watching.
The transition from "pills to plants" is a unification of chemical and clinical sciences. By applying the rigorous, peer-reviewed approach of the drug-checking movement [9-12] to the botanical heritage of the 1984 APPP, we are creating a unique safety net. The APPP is no longer just a catalogue of plants; it's a clinical tool for the Australian public, bridging the gaps between the laboratory and the bedside and the community. By standing on the shoulders of the 1984 pioneers, we are ensuring that the next generation of Australians -whether they are at a music festival or hiking in the high country - are protected by the most sophisticated toxicological tools at our disposal.

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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 UK 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.atu.ie/courses/bachelor-of-science-honours-forensic-investigation-and-analysis Accessed on 08/10/25

Academic Programmes

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 the module "Clinical Forensic Medicine" students study the role of the doctor in assessing persons in custody, assault victims, child maltreatment, 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: Forensic Medical Sciences MSc
https://www.qmul.ac.uk/postgraduate/taught/coursefinder/courses/forensic-medical-sciences-msc/

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) who is 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 practicals, 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
https://www.kcl.ac.uk/study/postgraduate-taught/courses/analytical-toxicology-msc
Accessed on 06/10/2025

This section on academic programmes in our peer review journal was prepared in October 2025. 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. Please make contact with the Editor-in-Chief if you want to mention a course for inclusion in a future issue.

Acknowledgements

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Website for IACFT Journal https://theinternationalallianceofclin.godaddysites.com

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