

Analytical Challenges and Interpretation of Novel Psychoactive Substances (NPS) in Postmortem Toxicology

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Abstract:

Novel psychoactive substances (NPS) are a new emerging and analytically challenging group of drugs which present significant challenges to postmortem forensic toxicology. Conventional targeted assays and immunoassays that are commonly used for drug detection usually do not detect emerging analogues due to the fact that they are continuously modified and tend to be pharmacologically different. The limited availability of certified reference materials and incomplete metabolic data and the short detection windows of many parent compounds further complicate the reliable identification. The ability to identify new or unknown compounds and metabolites has been enhanced by the use of high resolution mass spectrometry as well as suspect and non-targeted screening methods, but these must be confirmed with strict criteria and regular updates to library databases. It is just as difficult to interpret positive results. Because of post mortem redistribution, chemical instability, and matrix effects, in addition to the absence of known toxic and lethal concentration ranges, it is not possible to make a simple extrapolation between the measured level and the estimated antemortem exposure or toxicity. The use of a combination of drugs, which often occurs with NPS-related deaths, creates further pharmacodynamic and pharmacokinetic interactions that make attribution of cause difficult. Thus, results from a positive analysis should not be considered as evidence of lethal contribution, toxicological data should be interpreted in conjunction with autopsy findings, pathological observations, medical history and case circumstances. The review highlights the opportunities and challenges of new analytical innovations and interpretive principles that must be embraced in a flexible and multi-platform workflow and with an integrated approach to case assessment to ensure the correct medicolegal conclusion in an evolving NPS environment.

Keywords Novel psychoactive substances; Postmortem toxicology; High resolution mass spectrometry; Analytical challenges; Postmortem redistribution; Forensic interpretation; Polydrug use

1. Introduction

Novel psychoactive substances (NPS) are currently an important and constantly changing problem in modern forensic toxicology. It includes a wide variety of psychoactive substances that can have effects like those of controlled substances or unlike those of controlled substances. Importantly, the word novel does not imply a substance has just been invented; in general, it refers to the substances that have recently been introduced to drug markets or recently have been noticed due to their psychoactive properties. NPS have been marketed in the past with terms like “designer drugs,” “legal highs,” “research chemicals,” and other terms designed to set them apart from traditional street drugs. They are hard to recognize,

classify and analyze because of their chemical changes.

The relevance of NPS to forensic toxicology is because of the difference in time that it takes for new compounds to enter the market and the time needed for the analytical method to be validated. Changes of the molecule of a drug with a known psychoactive effect can result in a compound having significantly different pharmacological and toxicological properties. As a result, traditional toxicological testing methods are unlikely to detect emerging toxic chemicals. Analytical literature from the last few years pinpoints the high frequency of appearance of new analogues, the differences in potency, the short detection window, the challenges in metabolite identification, and the

limits on reference standards as significant hurdles to reliable NPS analysis (Krotulski et al. 2025).

The postmortem context adds to the complexities. In addition to determining if a substance was present, forensic toxicologists must determine the potential for impairment, toxicity, and death. Post mortem redistribution, decomposition, specimen-specific variation, analyte instability and lack of concentration ranges for many NPS can complicate interpretation. A positive analysis result is thus not necessarily associated with a role in death. Toxicology results, however, should be considered in conjunction with autopsy, pathological, circumstances of death, medical history and the presence of other substances. In recent reviews, NPS analysis of post mortems is specifically listed as an analytical and interpretive challenge (Matey et al. 2026a).

As NPS becomes even more diverse, forensic laboratories must use more flexible, increasingly sophisticated analysis strategies. While targeted approaches are still helpful when the suspected substance is known, high-resolution and non-targeted techniques can be helpful to identify unexpected substances and metabolites. The advances of such methods have contributed to the growth of forensic labs in being able to investigate previously unknown compounds.

1.1 Concept and Evolution of Novel Psychoactive Substances

Novel psychoactive substances has evolved with the shift in illicit drug markets, pharmaceutical chemistry, drug-control policies, and recreational drug use patterns. NPS are broadly known as psychoactive substances that are not necessarily subject to international drug control conventions but may have a detrimental effect on public health. The designation is therefore not entirely based on the novelty of the chemicals, but also on their recent appearance or increased availability. This is significant because some NPS might have been on the market for a long time before it went into the recreational market or forensic analysis.

The evolution of the NPS has been a process of modification of chemical structures on known psychoactive drugs in the past. Small molecular changes may result in analogues that exhibit modified receptor affinity, potency, metabolism and toxicity, but retain pharmacological properties

of existing drug classes. This phenomenon led to the emergence of substances designed to emulate and replicate cannabis, stimulants, opioids, hallucinogens, benzodiazepines and other psychoactive drugs. In response to regulatory oversight of specific chemicals or structural classes, manufacturers and illicit producers started to create other analogs. This has created the dynamic category of NPS in forensic drug analysis. There has also been a diversification of the chemicals used in NPS. Older discussions typically emphasized synthetic cannabinoids, synthetic cathinones, and designer stimulants while newer forensic laboratories are faced with an emergence of new classes of synthetic opioids, nitazenes, designer benzodiazepines, tryptamines, lysergamides, arylcyclohexylamines, and other structurally distinct compounds. A recent comprehensive review of postmortem NPS analysis highlights the need for chemical classification, as structural organization helps laboratories to set up targeted and wider analytical approaches.

Another hallmark of NPS development is the growing need for the quick dissemination of information from clinical toxicology, forensic laboratories, police and other law-enforcement agencies, poison centres and international monitoring systems. New chemicals can first appear in single clinical cases of intoxication or in unexplained cases of poisoning in the laboratory. Information can be utilized for laboratory database development and surveillance after its analytical identity and toxicological effects is recognized. But as new compounds emerge, it's necessary to keep analytical libraries and screening panels updated.

The development of NPS has therefore changed the nature of forensic toxicology from a focussed field to a more flexible analytical approach. The identification of substances not thought of in the first analysis is supported by high-resolution mass spectrometry, improved spectral libraries, metabolite investigation and non-targeted screening.

1.2 NPS: An emerging challenge in forensic and postmortem toxicology

NPS are a unique challenge in both forensic and postmortem toxicology due to the ability to appear before the necessary lab methods are developed to

detect and interpret them. In the case of conventional toxicological workflows, the targets of analysis, metabolites, reference materials and drug classes are well established. Newly emerging NPS, by comparison, may not have as much toxicological information or well-defined metabolism, nor be represented in traditional screening libraries, and may contain fewer or no certified reference materials. Therefore, it is important to keep in mind that a negative result on a routine toxicological screen does not rule out the possibility of NPS exposure.

The chemical structures and pharmacological properties of the NPS are diverse, making the analytical problem more complex. Others may suffer instability during analysis or storage, and some compounds may be present at levels that require use of very sensitive instrumentation. Some NPS may also be extensively metabolized, making the identity of the parent compound hard to find in biological samples. It can become necessary, in these cases, to identify the metabolites when the parent compound has a short biological persistence to establish exposure. In such cases, high-resolution mass spectrometry has proven quite useful, with the ability to aid in accurate-mass screening, retrospective data analysis, and the investigation of unexpected compounds (Ferrari Júnior et al. 2022).

Add to this uncertainty from post-mortem examinations. Post mortem processes may alter the distribution and concentration of drugs in the body. There might be differences in the levels of the analytes in the central and peripheral blood; autolysis and putrefaction may affect the level of the analytes in both biological matrices and the stability of the analytes. When trying to interpret the concentrations of a substance for which there is no ante-mortem therapeutic, toxic, or lethal range, such processes can be particularly problematic. Recent literature highlights the post mortem redistribution and lack of information on the post mortem concentrations-toxicity relationship as influences on NPS interpretation (Menéndez-Quintanal et al. 2024).

Polydrug exposure is still another challenge. NPS can be taken in combination with alcohol, prescription medications, illegal drugs or other NPS. The pharmacologic interactions can make it difficult to determine the clinical and pathological

effects of each compound. Therefore, the finding of an NPS should not be considered as a singular cause of death, but part of a larger investigation into toxicity.

These challenges have significant forensic implications. An NPS that goes undetected may result in inadequate toxicological conclusions, and a positive result may result in an incorrect claim of causation. Integrated analytical, pharmacological, pathological and circumstantial evaluation is thus necessary today in forensic toxicology.

1.3 Importance of NPS Detection in Medicolegal Death Investigation

The detection of NPS is becoming more relevant in medicolegal death investigations as an unexpected finding of such a substance may be helpful in determining the circumstances and mechanism of death. Forensic investigations are not only about the identification of toxins; toxicological analysis may be part of the greater picture and provide information on their possible role in death secondary to intoxication, poisoning, drug interaction, or other cause. This function is especially important if the autopsy results are not specific. Pathological examination may not always give a specific pattern of NPS intoxication, highlighting the need for thorough chemical toxicological analysis of recent reviews of NPS related mortalities (Benedetti et al. 2024).

NPS detection may also be used in the solutions of otherwise mysterious deaths. Many emerging compounds will not be detected in routine screening panels and cases involving NPS may be negative for commonly tested drugs initially. Advanced analytical testing can thus detect compounds which would not be found by any other method. Forensic laboratories have been able to increase their ability to identify low concentrations, unexpected compounds and metabolites by using LC-MS/MS and high-resolution mass spectrometry. These methods are especially helpful when there is no initial clues as to the identity of the particular substance.

Identification of NPS is also essential for the complete toxicological picture of a decedent. A substance can be found in conjunction with other drugs and its importance must be evaluated along with possible interactions with other drugs and circumstances of death. Literature regarding

fatalities involving NPSs notes that many of these cases involve the use of multiple substances, providing a good example of the benefits of toxicological analysis of more than one suspected substance.

NPS detection also plays a role in public health surveillance and forensic intelligence. Positive post mortem results can give information on substances that circulate in a population and can warn laboratories and health authorities of new threats concerning the toxicology of substances. The rapid spread of information about new chemicals can aid in the development of analytical methods, reference standards, spectral libraries and suitable screening procedures. Adverse NPS events have been reviewed and it has been stressed that the identification of these events in a timely fashion is relevant to broader public health and public safety responses, in addition to individual forensic cases (Mohr et al. 2022).

Finally, when there is reliable NPS detection the evidentiary basis of medicolegal conclusions is reinforced. But being found is not proof of NPS causing death. The toxicologist should take into consideration specimen type, analytical reliability, concentration, metabolites, post-mortem changes, and co-intoxicants, pathological findings, and case circumstances. Therefore, detection of NPS should not be considered a stand-alone diagnostic endpoint but should be seen as a part of an integrated medicolegal investigation.

Objectives

- To critically examine the principal analytical challenges associated with the detection, identification and quantification of novel psychoactive substances in postmortem biological matrices, including limitations of conventional methods, scarcity of reference standards, metabolite identification and the role of advanced chromatographic–mass spectrometric techniques.
- To evaluate the interpretive complexities of NPS findings in medicolegal death investigations, with particular emphasis on postmortem redistribution, analyte stability, polydrug interactions and the necessity of integrating toxicological results with pathological and circumstantial evidence.

2. Classification and Toxicological Characteristics of Novel Psychoactive Substances

Novel Psychoactive Substances (NPS) are a diverse group of compounds both chemically and pharmacologically. They are usually categorized by structural properties, receptor activity, major psychoactive effects, or by their resemblance to a known drug class. The major classes of synthetic drugs are synthetic cannabinoids, synthetic opioids, synthetic cathinones and other stimulants, serotonergic hallucinogens, designer benzodiazepines, and dissociative substances. This classification is especially important in forensic toxicology, as it affects the clinical effects to be expected, target analytes, analytical methods, and contribution to death. Pharmacological profiling studies showed that NPS can interact with a variety of molecules, including the cannabinoid receptor, the opioid receptor, the monoamine transporters, the serotonin receptor, the GABA-A receptor, the NMDA receptor, etc (Liechti et al. 2021).

The toxicity properties of NPS are very diverse even within the same general classes of chemicals. Structural modifications may result in changes in receptor affinity, intrinsic activity, metabolic pathways, biological half-life, and potency. Therefore, the toxicity of close analogues cannot be assumed to be similar. This is a problem if previously developed toxicological data are applied to another compound. It is particularly important in the interpretation of the results of toxicology performed in the postmortem setting, where concentration based interpretation depends on the pharmacokinetics, postmortem redistribution, tolerance, characteristics of the specimen, and interactions with other substances.

For instance, synthetic cannabinoids are a class of drugs that are strong agonists of the cannabinoid receptors, and can be toxic to the cardiovascular, neurological, psychiatric, and systemic systems. Another important group is synthetic opioids since some of them have extremely high μ -opioid receptor affinity. The stimulant activity of synthetic cathinones typically results from their interaction with the monoamine transporters, while the serotonergic mechanisms are the main source of the stimulant activity of tryptamines and related hallucinogens. The main mechanism of action of designer benzodiazepines is to increase

GABAergic transmission, and dissociative NPS are linked to antagonism of the NMDA receptor.

2.1 Synthetic Cannabinoids

Synthetic cannabinoids are also better known as synthetic cannabinoid receptor agonists (SCRAs) and are a structurally diverse group of NPS that mimic the effects of Δ^9 -tetrahydrocannabinol (Δ^9 -THC). The toxicological significance is due to the fact that many SCRAs differ from natural cannabis. Many synthetic cannabinoids have strong activity at the CB1 and CB2 receptors, and can have significantly different pharmacological activity, while Δ^9 -THC is a partial agonist at the cannabinoid receptors. This is one of the reasons why they can be toxic in unpredictable ways and makes it difficult to find general toxic levels (Giorgetti et al. 2020).

Exposure to SCRA can produce a wide range of toxicological effects. Acute intoxication can include agitation, anxiety, confusion, hallucinations, changes in consciousness, tachycardia, hypertension, convulsions and neurological and cardiovascular effects. Severe cases can include hyperthermia, rhabdomyolysis, renal injury, respiratory abnormalities, and/or cardiovascular collapse. The exact clinical picture depends on the compound, the dosage, the route of exposure, a person's tolerance, and multiple exposures. Therefore, results from one SCRA cannot be extrapolated to the other category.

One of the large-scale forensic problems is the lack of established toxic and lethal concentration ranges. In a systematic review of death associated with SCRAs, typical toxic ranges were found to be inadequate and cautioned against the interpretation of toxicology results. When a synthetic cannabinoid is found in post mortem concentrations, it should be evaluated in conjunction with the autopsy findings, circumstances, other toxicological tests results, and available clinical information.

Another factor important in a forensic examination is the quick turnover of compounds in a street market. Structurally modified analogues can also be found when SCRAs are subject to specific control. This makes it challenging to laboratories as traditional screening methods might not pick up on novel compounds. Moreover, many SCRAs are metabolised extensively, and metabolites can be a

key clue of exposure if the parent compound is not being detected or is at low concentration.

Based on the post mortem, synthetic cannabinoids would thus need a sensitive analytical approach and careful toxicological evaluation. While their detection may be very informative, analytical positivity should not be taken as an automatic indication of a cause of death.

2.2 Synthetic Opioids and Nitazenes

Synthetic opioids are a significant class of NPS due to their ability to induce strong opioid effects, including analgesia, sedation, respiratory depression and respiratory failure, which can be fatal. Fentanyl analogues have already drawn significant forensic interest, but another new class of non-fentanyl synthetic opioids are emerging: nitazenes. Nitazenes share a 2-benzylbenzimidazole structure and are sometimes referred to as isotonitazene, metonitazene, protonitazene and analogues of these. It has been shown that their pharmacological actions are mainly linked to μ -opioid receptor activation (Vandeputte and Stove 2025).

It is important to note that a few nitazenes are very potent opioids, meaning that they are especially noteworthy. Pharmacological studies in the past few years have suggested that some analogues have higher μ -opioid receptor activity. This implies that very small doses can produce significant opioid effects, but it is important to note that the potencies of individual analogues vary greatly. Clinical toxicological signs may consist of severe sedation, reduced breathing and consciousness, coma, and death.

They are made forensic important by the fact that they are hard to detect by normal toxicological means. However, the inability to detect routine use of an opioid does not rule out the possibility of nitazene exposure because conventional opioid immunoassays do not recognize all synthetic opioids that are structurally different. Individual analogues and their metabolites may need to be identified using specialized mass-spectrometric techniques and/or suitably constructed analytical libraries. The increasing variety of nitazene compounds makes it a challenge to monitor in the laboratory and develop new methods (Verbeek and Brinkman 2025).

Another challenge in interpretation of nitazenes in post mortem cases is the frequent use of them together with other substances. A decedent can have been exposed to more than one opioid, stimulant, benzodiazepines, alcohol or another NPS. Therefore, it is important to measure the full toxicological profile of a nitazene, not just the concentration present, to identify the nitazene.

The appearance of nitazenes thus illustrates the ability of NPS to generate a pharmacological challenge, analytical ambiguity, and interpretive uncertainty. The focus of current literature is on the need for continuous monitoring, enhancement of analytical detection, characterization of metabolites, and the collection of postmortem toxicological data to further advance understanding of this evolving class.

2.3 Synthetic Cathinones and Other Stimulants

Synthetic cathinones are one of the primary classes of NPS with stimulant properties and are structurally similar to cathinone, the psychoactive ingredient in *Catha edulis*. They are structurally similar to amphetamines and may act as stimulants and in a few instances entactogens. Some of these examples are mephedrone, methylone, methcathinone, MDPV, α -PVP, α -PHP, and analogues. The activity, potency, duration, metabolism, and toxicity of the transporter can be greatly modified by structural changes about the cathinone scaffold(Chen, Zhou, and Lai 2024).

Synthetic cathinones exhibit their major pharmacological effects via effects on monoamine transport systems. Individual compounds can either inhibit the reuptake of dopamine and norepinephrine or increase the release of monoamines, or influence serotonin transmission, depending on their molecular structure. These mechanisms can lead to hyperactivity and euphoria, but can also lead to overactive sympathetic responses. Symptoms of toxicology may include agitation, anxiety, hyperthermia, hypertension, tachycardia, seizures, psychosis and cardiovascular reactions. Cardiotoxicity has been discussed recently as a significant side-effect of exposure to synthetic cathinones(Groenewegen et al. 2024).

The cathinone chemical structure is a key factor in the forensic issues. Small modifications can yield new analogues with novel pharmacological

properties that have sufficient structural similarity to fall into the same general class. Therefore, the analytical laboratories need to regularly update the reference materials, the spectral libraries, and the target panels. The extraction techniques have also been the subject of a systematic review, highlighting challenges involved in biological matrices, and the potential for low levels of synthetic cathinones(Gu et al. 2024).

Interpretation of post mortem samples is difficult, as the concentrations determined by fatal intoxication can vary widely. A recent study of α -PVP, α -PHP and α -PiHP that examined post mortem samples found a broad range of concentrations in fatal cases, and found that other drugs were also commonly being abused. The results support the need to take into account tolerance, co-exposure, specimen type and pathological finding as opposed to applying a single concentration threshold to determine toxicity(Kriikku and Ojanperä 2024).

Other stimulant NPS are substituted amphetamines, aminoindanes, benzofurans, and piperazine derivatives. These compounds have stimulant effects but they act upon distinct molecular targets and have different toxicological profiles. Therefore, they can be broadly classified as 'stimulants' but cannot be specifically interpreted forensically.

2.4 Novel Hallucinogens, Tryptamines and Lysergamides

Novel hallucinogens include a number of chemically distinct NPS that commonly affect perception, cognition, mood, and sensory processing. These include substituted phenethylamines, tryptamines, and lysergamide-related compounds. Within these, tryptamines are structurally similar to serotonin, and include naturally occurring tryptamines and synthetic derivatives. They have psychoactive properties which are primarily linked to serotonergic receptor activity, particularly the agonism at the 5-HT_{2A} receptor(Malaca et al. 2020).

The tryptamines can cause intense changes in perception and consciousness. Symptoms can range from seeing and/or hearing things that aren't there, to changes in time perception, anxiety and agitation, and behavior changes, depending on the compound and exposure situation. Autonomic

manifestations like tachycardia, hyperthermia, hypertension or seizures may also occur with some compounds. The toxicological profile is not a uniform entity because substitutions on the tryptamine structure can make the receptor selectivity, potency, duration and metabolism different.

One of the biggest forensic challenges is lack of toxicological data on many newer chemicals. Tryptamines are not routinely tested in every toxicology laboratory, and may therefore be underdiagnosed in intoxications and deaths. Several compounds have limited information available on their mechanisms of action, pharmacokinetics, toxicity, analytical detection and metabolites, according to a dedicated review. It also indicated that liquid chromatography (LC) coupled to tandem mass spectrometry (MS) is an emerging analytical tool for the detection of tryptamines and their metabolites.

Lysergamides are another class of serotonergic hallucinogens with a similar structure to lysergic acid diethylamide (LSD). Structural changes can result in compounds with a different effect of action and duration of effect. For sensitive mass-spectrometric methods are important in forensic analysis, as many of these substances can be present at low concentrations, and may have limited references.

Hallucinogenic NPS should be interpreted with special care in cases of post-mortem. Simply finding a serotonergic hallucinogen in someone's body does not prove that the drug caused the death, particularly if other drugs are found. Furthermore, concentrations of toxic and lethal effects are not known which restricts quantitative interpretation. Therefore, it is advisable to interpret analytical results in relation to the circumstances of the case, autopsy results, metabolites and co-intoxicants.

The growing number of serotonergic NPS thus poses a challenge both analytical and pharmacological. New reference standards, spectral libraries, metabolite information, and comprehensive screening methods are needed to enhance detection and interpretation.

2.5 Designer Benzodiazepines and Dissociative Substances

Designer benzodiazepines are NPS with pharmacological properties that are broadly similar

to the standard benzodiazepines. They have been introduced onto recreational drug markets in a modified benzodiazepine structure, prior to sufficient information on pharmacokinetics, toxicity and clinical effects. Clonazepam, flualprazolam, flubromazolam, etizolam, diclazepam, phenazepam and related compounds have been discussed in the forensic literature. They have a major pharmacological effect by increasing GABAergic neurotransmission by acting upon GABA-A receptors, leading to sedative, anxiolytic, hypnotic, and muscle-relaxant properties(**Brunetti et al. 2021**).

One of the reasons designer benzodiazepines are toxic is because they vary. Changes in activity, potency, half-life, and metabolism and/or active metabolites can significantly alter clinical effects. Intoxication may include somnolence, incoordination, confusion, respiratory depression and coma. Combined with other central nervous system depressants (such as alcohol or opioids), the risk of severe toxicity may increase with designer benzodiazepines.

Another significant challenge is analytical detection. Numerous designer benzodiazepines are not found on the common laboratory panels, and their metabolites could be different from those of common benzodiazepines. Therefore, exposure would not be detected through routine screening unless the method was cross-reactive or the lab used targeted or high resolution mass spectrometric screening. Inadequate validated analytical methods, as well as lacking pharmacokinetic and toxicological data for numerous compounds, have also been pointed out in the published literature.

Dissociative NPS are a distinct class of drugs, such as arylcyclohexylamines, including methoxetamine and analogues of PCP. These compounds usually have dissociative effects by being antagonists of NMDA receptors. The acute effects can range from altered consciousness to perceptual changes, lack of coordination, agitation and even psychotic symptoms. There are also some compounds that have been linked to cardiovascular, neurological, renal or other systemic toxicity(**Hassan et al. 2017**).

The interpretation of both groups is difficult because of the high incidence of co-presence of substances. Concentration is not always the only toxicological parameter that can be used to

determine the toxicological significance of a designer benzodiazepine or dissociative in postmortem cases. The type of specimen, post mortem changes, tolerance, active metabolites and other CNS active substances should be taken into account. There is significant inter-class pharmacological variability in these categories, therefore, compound-specific rather than class-specific interpretation should be provided.

2.6 Pharmacological Diversity, Potency and Polydrug Use

The most important toxicological feature of NPS is their variety of pharmacology. The chemical structures of NPS can interact with a variety of receptors, transporters, ion channels and neurotransmitter systems whereas traditional drug classes usually have more widely accepted mechanisms of action. Stimulant NPS can alter the actions of dopamine, norepinephrine, and serotonin; many hallucinogens target serotonergic receptors; synthetic cannabis acts primarily through cannabinoid receptors; synthetic opioids target opioid receptors; many designer benzodiazepines act on GABA receptors; and dissociatives interfere with the actions of NMDA receptors (Liechti et al. 2021).

This diversity will have direct implications with regards to potency and toxicity. Large differences in receptor affinity and intrinsic activity are possible between closely related analogues, using structural modification. So, toxicological behaviour cannot be predicted by chemical similarity. Nitazenes are a special case as a number of analogues show a very high opioid activity and synthetic cannabinoids also show a wide range of receptor activity and toxicity.

Potency also poses analytical challenges in forensic toxicology. Those compounds that are highly active can cause potentially high levels of impact at low concentrations that may not be analytically validated without proper case context and might be considered background or contamination. However, a low measured concentration should not be regarded as clinically insignificant as concentration will depend on dose, timing, metabolism, specimen selection, redistribution, tolerance and analytical stability.

Interpretation is further complicated by the fact that some individuals may be using multiple drugs.

NPS are often taken in combination with other NPS, illicit drugs, prescription drugs or alcohol. A pharmacodynamic interaction may cause a greater toxicity, especially if the two drugs have similar effects on the central nervous system, cardiovascular system, or respiratory system. Co-exposure was also highlighted in recent post-mortem studies as a factor that was crucial in the context of NPS-related deaths and shows the complexity of determining death from a single substance detected (Benedetti et al. 2024).

It is important to take the whole toxicological profile into account when interpreting the data from a forensic perspective and certainly not just one concentration or compound detected. Factors such as pharmacological class, known potency, metabolites, tolerance, specimen type, postmortem redistribution, co-intoxicants, pathological findings and circumstances of death are all important considerations.

3. Innovations in Analytical Detection and Identification of NPS

3.1 Chromatographic and Mass Spectrometric Approaches

The chromatographic and mass spectrometric methods are the main bases used for the identification of NPS in forensic toxicology. The significance of their role is related to the requirement to distinguish structurally similar materials in chemically complex biological matrices. The separation of individual analytes is achieved by chromatography, while mass spectrometry supplies molecular and structure information by measuring the mass to charge ratio and the fragmentation patterns of the molecule. The combination thus provides a superior degree of specificity than screening based on pharmacological class or immunochemical cross-reactivity.

GC-MS is a mature technology in forensic toxicology that is used for the reproducible separation by chromatography coupled with the characteristic electron-ionisation mass spectra. If a substance is known and exists in known libraries, spectral libraries can aid in identification. However, for certain NPS, thermal instability, volatilization requirements and derivatization requirements limit the applicability of the

technique. There are also times in which the introduction of chemically modified substances occurs rapidly enough that reference spectra are not available, which diminishes the value of a library dependent identification.

LC-based approaches have therefore gained in importance with increasing time. LC can be used to analyze polar or thermally labile (or otherwise inappropriate) compounds. A tandem mass spectrometry (MS/MS) system combined with LC allows for selective monitoring by precursor and product ion which is especially appropriate for multi-analyte targeted assays. This flexibility has enabled the development of analytical methodology for a number of NPS classes at once, as opposed to the development of an analytical method for each individual NPS (Feigel, Adamowicz, and Wybraniec 2024).

Another great benefit of LC–MS/MS is its ability to be used for compounds at low concentrations. Some NPS have high pharmacological activity, so that toxicologically relevant exposure does not necessarily result in high analytical concentrations. It is therefore critical to use sensitive instrumentation for reliable detection. However, the ability to utilize targeted LC–MS/MS is highly reliant upon the compounds chosen for inclusion in an analytical panel and the presence of suitable transitions and standards. A new analogue can then, when the method is updated, be analytically invisible.

The modern scientific work thus confirms an auxiliary nature, not replacement one, of the relationship between chromatographic platforms. GC–MS is still helpful for the analysis of appropriate analytes and identification by libraries, and LC–MS/MS has significant benefits for broad targeted analysis of chemically diverse NPSs. These limitations have driven the development of high-resolution mass spectrometry that can give a more complete molecular picture of a substance and enable the study of an unknown substance that may not have been thought of during a method's design (Remane, Wissenbach, and Peters 2016).

3.2 LC–MS/MS and High-Resolution Mass Spectrometry

The analytical investigation of NPS is a significant advancement in the field of mass spectrometry, with LC–MS/MS and high-resolution mass

spectrometry (HRMS) being the two key innovations. In the field of targeted identification, LC–MS/MS is becoming of great value as the tandem mass spectrometers can be used to selectively monitor desired molecular transitions. This attribute enables laboratories to develop analytical panels for specific NPS and their metabolites. This method is particularly suited when the substance has been previously identified and tested and validated quantitative or qualitative procedures exist.

HRMS is another approach to analytical need: It is used to study compounds which might not have been expected in the methods development process. Accurate-mass measurement can yield elemental-composition data and significantly improve the amount of data available for structural elucidation. Technology such as time-of-flight and Orbitrap can acquire wide mass ranges which then can be analyzed after the acquisition. Information gathered in one analysis then may prove to be valuable for a subsequent analysis of a previously unrecognized NPS or when a new NPS is discovered and included in a reference database (Klingberg et al. 2022).

One of the most significant attributes of HRMS is its ability to support data independent acquisition and other "umbrella" acquisition strategies. These methods do not limit the number of transitions that are acquired, but rather provide a vast amount of mass information to be interrogated at a later time. This capability is very applicable to NPS as the drug market evolves faster than the conventional targeted analytical panels can be updated. HRMS thus provides a more flexible analytical approach where legacy data can be re-evaluated as new information on new compounds becomes available. In spite of these benefits, HRMS should not be considered as always superior to tandem quadrupole MS; in some cases, sensitivity to specific analytes, quantitative performance, speed and the cost of the instruments may be problematic. However, HRMS can also have problems with some structural isomers which yield similar mass-spectral properties. Therefore a choice of platform should be made on the basis of the analytical question, not just the novelty of the platform (Pasin et al. 2017).

Integrated analytical workflows are therefore well supported by the current literature, in which

targeted LC–MS/MS and HRMS complement each other. Targeted methods are very efficient at confirmation of known substances, and HRMS enables for more extensive screening, retrospective analysis and investigation of unknown substances. This pairing is especially useful in forensic toxicology where the identity of an NPS may not be known initially and then analytical data must be related to toxicological interpretation(Wille et al. 2022).

3.3 Targeted, Suspect and Non-Targeted Screening Strategies

The progressive expansion of the scope of analytical toxicology can be seen in the development of targeted, suspect and non-targeted screening strategies. In targeted screening, a list of substances is defined and the analytical signals of these substances are searched. When the substance(s) of interest are already known, this approach is very efficient as the analytical system can be optimized for the expected chromatographic and mass-spectrometric properties of the substance(s) of interest. Targeted approaches can thus be especially helpful for routine confirmation and quantitation of known NPS(Feigel, Adamowicz, and Wybraniec 2024).

One of the chief drawbacks of the targeted approach is that it relies on the prior knowledge of the target. If there is no compound in the analytical panel, then it is not likely to be identified, even if the instrument can detect it. This generates a significant gap in NPS toxicology as the modification of the structure could result in the emergence of a new substance, which may differ significantly to the compounds monitored in the past. As a result, ongoing targeted panel expansion is the need of the hour in the NPS market(Graziano et al. 2019a).

Suspect screening is a middle ground approach. Instead of the full analytical criteria for all possible compounds, it relies on data related to suspected compounds, molecular formulas, accurate masses, predicted fragments, or other spectral properties. This enables laboratories to look for substances that were not originally targeted in the workflow, but are present in existing datasets at a high resolution. Consequently, suspect screening can broaden analytical scope with a level of prior chemical knowledge(Wille et al. 2022).

Non-targeted screening is a more comprehensive approach that tries to identify chemicals that are not anticipated. HRMS is ideal for this application because it allows for the acquisition of a spectrum over a wide mass range and then the use of the spectra for library searching, molecular formula identification, fragmentation pattern analysis and computational structure elucidation. These methods can aid in the identification of new analogues and metabolites that were not previously known. This is where their importance is especially notable given that there can be situations where new compounds are introduced before full reference data can be obtained.

But there are also interpretive challenges with broader screening. The discovery of an unanticipated molecular property does not necessarily mean that it is identified, pharmacologically active or of forensic interest. Careful consideration of accurate mass, chromatographic behaviour, fragmentation evidence and available reference information is necessary for candidate identification. Therefore, targeted, suspect and non-targeted approaches must be seen as complementary approaches to analytical investigation and not as competing technologies. Their integration offers a flexible structure to maintain detection capabilities with the ongoing changes in NPS chemistry.

3.4 Reference Standards, Metabolite Identification and Analytical Validation

The basics of reliable NPS identification include reference standards, which are essential for analytical confirmation, which typically involves comparing observed characteristics with information relating to an authenticated substance. Certified reference materials are often not available when a compound first enters the NPS, as they tend to arise suddenly. This leaves a big difference between identifying a chemical feature and making a defensible identification. The shortage of reference material can therefore cause a delay in method development, expansion of the spectral-library and routine forensic confirmation(Jousselin et al. 2026).

Another crucial element of NPS analysis is metabolite identification. Many parent substances are extensively biotransformed, and may thus not be as useful analytically as one or more

metabolites. Understanding of metabolic pathways helps to pinpoint suitable markers and enhance the chances of detecting exposure. However, little information about the metabolism and toxicokinetics of newly emerging NPS is available. It is noted that mass-spectral libraries are important to the improvement of detection capability, especially those containing metabolites of relevance(Wille et al. 2018).

Reliability of an analytical method is established using analytical validation. Selectivity, sensitivity, calibration, accuracy, precision, recovery, matrix effects and stability are all important features; some of these features are mandatory for the qualitative analysis procedure, while others may be necessary for quantitative, targeted or comprehensive analysis procedures. Uncertainty in measurement and analytical identification criteria are also important considerations for forensic applications.

A special consideration in the context of matrix effects is the possible interference from endogenous components of biological samples that can affect the ionisation efficiency and thus the response to the analytical method. The method should therefore be demonstrated to be valid for the intended analytical context, through appropriate validation. This is especially critical for NPS as structurally different substances could exhibit different behavior in the same analytical platform. Validation should therefore be considered to be a continuous quality assurance process, not merely a technical requirement.

There is also a need to highlight the need for internationally agreed identification and validation principles. The need to include wide-ranging HRMS workflows in analytical toxicology is on the rise, and it is important to define defensible criteria to distinguish between reliable identification and tentative structural assignments. This is important in a forensic context as analytical conclusions can impact on the medicolegal interpretation. The ongoing development of reference-material resources, metabolite libraries, coupled with the development of a standardized validation framework, is therefore crucial to further improve the reliability and comparability of NPS toxicology.

4. Analytical Challenges in Postmortem NPS Toxicology

4.1 Rapid Emergence, Structural Modification and Limited Reference Standards

The rapid emergence of new drugs into the recreational drug market is one of the characteristic analytical challenges of NPS. NPS are often engineered in a structural modification of an already known psychoactive substance, resulting in a chemical structure and pharmacological property that is typically different from the parent compound. Small changes in molecular structure may alter the chromatographic retention, ionization and fragmentation characteristics, metabolism, as well as the analytical detectability. Thus, there is no guarantee that an analytical method that is optimized for one substance will be suitable for reliable detection of a structurally related analogue. Continued introduction of modified compounds then puts a significant strain on forensic laboratories to keep analytical libraries and screening methods up to date.

The structural diversity also makes it hard to predict what compounds should be tested as part of a routine toxicological test. Standard targeted screening relies on a set of pre-defined analytes and analytical data. If a newly emerging NPS is not found in this list, it could pass through the net even if the lab has high-end equipment. In forensic toxicology, it is important since negative analytical results do not always mean that the substance in question was not involved. Rather, the negative outcome could be due to the limitations of the analytical technique(Stephenson et al. 2024)(Rygaard et al. 2023).

The lack of certified reference materials (CRM) contributes to the difficulty of identification as well. Usually, enough information about the expected chromatographic and mass-spectral behaviour of the compound will be required to be able to make reliable confirmation. Newly found NPS might not have commercial or certified reference materials available, making it more challenging for laboratories to develop definitive identification methods. Limited reference materials can also slow the development of quantitative techniques and the growth of reference spectral libraries(Peters and Meyer 2021).

Another problem is the lack of detailed toxicological data on newly developed chemicals. Information concerning pharmacokinetics, metabolism, tissue distribution, stability and toxicity may be considerably less developed than for established drugs. This causes a disconnect between analytical recognition and toxicological interpretation. Even if an emerging NPS is identified and validated, it may take some time before appropriate concentration ranges and toxicological benchmarks are established, and therefore the forensic significance may not be known(Abdelaal et al. 2024)(Pélissier-Alicot et al. 2003).

The literature thus confirms the need for continued growth of analytical libraries, collection of reference materials, and information sharing networks. While this can't be completely solved, high-resolution mass spectrometry and suspect or non-targeted approaches can assist the laboratory to study compounds outside of the targeted panels. However, it is still necessary to have proper supporting evidence to be able to make a definitive identification. The persistent nature of the ongoing emergence and evolution of NPS, however, means it is a challenge, not a problem that can be solved with one analytical technology.

4.2 Post mortem Redistribution, Degradation and Stability

The main cause of difficulty in interpreting drug concentrations post mortem is postmortem redistribution (PMR). It relates to the changes occurring in the distribution and concentration of drugs in the body after death. Once dead, physiological barriers will no longer be functioning, passive diffusion or cadaveric processes such as tissue breakdown may alter the location and concentration of drugs. This means that the concentration measured in a forensic analysis could be very different from the concentration around the time of death.

Redistribution depends on the properties (physicochemical and pharmacokinetic) of individual substances. The chances of a compound crossing from one organ to another in the body after death will be affected by lipophilicity, ionization properties, protein binding, volume of distribution and affinity for tissues. Pharmaceuticals that are concentrated at relatively

high levels in organs like the gastrointestinal tract, myocardium, lungs, and liver can diffuse to the surrounding tissue or blood. There may be significant differences in post mortem concentrations, depending on the site of the processes(Yarema and Becker 2005)(Kennedy 2015).

NPS cause further uncertainty as they are often poorly characterised physiochemically and pharmacokinetically. Available information suggests that several NPS groups might be susceptible to redistribution, with little available information for many newly introduced compounds. Thus, some redistribution patterns, based on traditional drugs, cannot be used to interpret the concentration of an NPS.

A related, but separate issue is chemical stability. NPS can be degraded, after they have died, by hydrolysis, oxidation, reduction and other chemical reactions. Decomposition may also change the parent compound or create new chemical products as a result of residual enzymatic activity or microbial processes. The measured concentration may thus represent both the initial exposure and changes that may have taken place between the time of death and the time of analysis. This instability can lead to underestimation of the parent substance or the presence of a metabolite or degradation product that can interfere with identification or be more analytical than the parent drug.

These processes can be further affected by the post mortem interval and environmental conditions. Analyte stability can be influenced by the time between death and toxicological evaluation, storage conditions and decomposition. Therefore, interpretation should take into account the chemical nature of the NPS as well as the postmortem interval. The literature places great importance that posts mortem levels should never be assumed to be equivalent to antemortem levels(Baba et al. 2026).

Because of these factors, the PMR and stability is especially significant when attempting to make an association between an NPS and fatal intoxication. Forensic interpretation should not solely be based on the concentration, but also take into account distribution, known physicochemical behaviour, stability, and other toxicological information. A significant challenge for developing reliable

interpretive frameworks is the lack of comprehensive PMR data for many NPS.

4.3 Unknown Metabolites, Matrix Effects and Analytical Interferences

Identification of NPS metabolites is an important but challenging part of postmortem toxicology. There are some psychoactive drugs that are highly metabolized, and metabolites can still be detected in biological samples after the parent compound has decreased significantly. However, metabolic pathways may not be very well characterized for newly emerging NPS. This means that analytical procedures and what chemical properties to look for as evidence of exposure is not always clear.

Targeted analytical approaches can sometimes be problematic for unknown metabolites. The parent drug and a few known metabolites can be used as the basis for a conventional assay. When a new compound is discovered, it could undergo an unexpected metabolic transformation that will cause the biomarker to not be included in the analytical method. Therefore, the presence of a metabolite in a specimen does not necessarily mean the exposure of the person was detected. One reason for this limitation is that modern forensic toxicology increasingly uses more general mass-spectrometric methods that can be used to study unexpected molecular properties.

Another significant analytical problem is matrix effects. In biological samples, there are natural compounds, proteins, lipids, salts and other substances that can affect the separation in chromatography and ionization in mass spectrometry. The components may either suppress or amplify analytical signals which could lead to an apparent response of an analyte. This may be complicated in post mortem specimens as decomposition yields other chemicals and breakdown products(**de Campos et al. 2022**).

Structurally similar compounds can also cause analytical interference. Groups of analogues that have related molecular structures are often found in NPS. NPS are often found to contain groups of analogues with similar molecular structure and sometimes closely related fragmentation behaviour. It is essential that these compounds are well separated chromatographically and that there is good mass-spectral evidence for distinguishing them. If the substance is not sufficiently selective,

it may be difficult to identify because there are structurally similar compounds or substances that are present in the body. This is especially true in low concentrations where the analytical signal is near the background.

There are also decomposition products, which add an extra layer of complexity. Compounds may be present in the tissues that are not normally detected and can interfere with an analysis or may be present during the postmortem period which can complicate interpretation. The problem therefore cannot be confined to just NPS but the overall chemical environment of the post mortem specimen must be taken into account. Sometimes, alternative matrices may contain additional information as other tissues or fluids may be more or less susceptible to degradation or interference.

The literature thus confirms the need to use analytical methods that enable analysis of known or unknown compounds. Advanced data-processing methods and metabolite information together with high-resolution mass spectrometry and spectral databases can enhance the identification of unknown compounds. However, the distinction between tentative detection and definitive identification has to be made. Analytically interesting molecular features might need further structural evidence before being reported as confirmed NPSs(**Peters and Meyer 2021**).

5. Interpretation of NPS findings in post-mortem toxicology

5.1 Interpretation of Postmortem Concentrations and Distribution Patterns

Post-mortem NPS concentrations should be interpreted with care as post-mortem concentrations may not reflect in vivo concentrations. After the blood ceases to circulate, drugs can migrate to organs, tissues and blood. Typically, this phenomenon is referred to as postmortem redistribution (PMR); this is affected by physicochemical properties, tissue affinity, volume of distribution, lipophilicity, pKa and the anatomical site of drug reservoirs. Therefore the numerical concentration found in a post mortem specimen is the result of both the exposure and some post mortem processes.

Particular importance of pattern of distribution between central and peripheral blood. Blood from near organs with large drug reservoirs might be more affected by concentration changes after death due to diffusion. The peripheral blood is more likely to be useful than any other blood source for interpretation because it is less directly influenced by organ release. However, peripheral specimens are not totally immune from postmortem changes. Blood concentrations must then be interpreted as derived from a specific anatomical source and not as all blood concentrations being the same (Borra et al. 2026).

There is also a context window of information that can be obtained from the relation of tissue and blood concentrations. Comparative approaches, using peripheral blood and tissues like liver, can help to determine if redistribution may have taken place. The distribution ratios should not be used as a universal diagnostic threshold however, as they are distribution specific and influenced by various biological and post mortem variables. Therefore, a number of literature-based markers of central-to-peripheral relationships as well as liver-to-peripheral relationships have been explored as tools to aid interpretation rather than as measures of toxicity (Zuccarello et al. 2026).

Another key factor to consider is that NPS concentration may vary with time. The quantity of parent substance or metabolites may be altered by autolysis, putrefaction, diffusion and residual metabolic processes. Thus, the time of death to specimen collection may affect the analytical result. This time dimension can create challenges for direct application of standard pharmacokinetic assumptions for postmortem toxicology.

For NPS, the issue is further complicated because there is a scarcity of information on redistribution specific to a given compound. There is limited information regarding tissue distribution and postmortem behaviour of many substances, especially those that are relatively new. Therefore, when interpreting, one should not presume that an NPS will act like another compound simply because the two share a common pharmacological class. Information on structural similarity may be helpful but will not confirm identical behaviour in postmortem.

5.2 Toxicological relevance of NPS in mortal cases

One of the most challenging parts of the forensic interpretation is establishing the toxicological relevance of a NPS found postmortem. An NPS found in a post mortem specimen does not necessarily mean that exposure has occurred, but it does mean someone was exposed. A substance can be found and it may not be the cause of death, it may be part of a toxic process that is fatal, or it may be a finding without any toxic significance. This distinction is relevant for NPS as the toxicological knowledge of many compounds is significantly less developed than for conventional drugs.

There is significant variation between published reviews of NPS-related deaths in the circumstances and toxicology of fatal cases. All synthetic cannabinoids, synthetic opioids, synthetic cathinones, and other NPS have been linked to permanent harmful side effects and fatalities. The occurrence of deaths, however, does not create a consistent concentration-response relationship for the entire NPS class. The potency, receptor activity, metabolism and toxicity of individual compounds in the same general category can be quite different (Matey et al. 2026b) (Concheiro et al. 2018) (Kraemer et al. 2019).

This is an example of an interpretive problem: synthetic cannabinoid receptor agonists. Evidence from systematic review has suggested that it is difficult to establish typical toxic range for these compounds. Therefore, the entire forensic context should be considered when interpreting rather than comparing to a universal lethal concentration. When assessing a possible contribution to death of the NPS, autopsy findings, circumstances, co-exposure and the analytical reliability of the detected substance are all important.

A second problem is that of concentration versus potency. A low numerical value does not necessarily mean that a result may be insignificant, as some NPS may have significant physiological effects at relatively low concentrations. On the other hand, a relatively high postmortem concentration does not guarantee fatal intoxication, as concentrations can be altered by redistribution, tolerance, metabolism, etc. However, a relatively high concentration does not necessarily mean that a person died from intoxication, as concentrations

may be affected by redistribution, tolerance, metabolism, and other postmortem factors. This can lead to potentially misleading simple numerical ranking of NPS concentrations.

Therefore, a distinction must be made between the presence and probable contribution, and demonstrated causation when interpreting fatal NPS cases. The toxicological finding is more likely to be a cause of death when it is consistent with the known pharmacology of the drug, the clinical or circumstantial history supports that finding, there is pathological evidence of exposure which also fits the pharmacology of the drug, and alternative causes of death have been eliminated. However, when data on concentration are limited or several alternative explanations are possible, there should be a substantial degree of uncertainty (Drummer et al. 2013).

Literature-based fatality reviews are therefore useful, as they offer comparative data from past cases reported in the literature. However, these compilations should not be used as concentration standards. Their primary significance is that they can help to illustrate trends in toxicity, related materials, pathological results, and ranges of concentrations that may be reported in cases that should be considered but can never supplant the forensic judgment of the individual case.

5.3 Polydrug Use and Interaction-Related Interpretive Challenges

Polydrug use is an important factor to take into account when interpreting NPS related deaths. People may also use other NPS, be using other conventional illicit drugs, prescription medicines or alcohol while exposed to NPS. Several substances may be detected in toxicology after death and these do not indicate that the presence of each one played an equal role in the death. Forensic problem is to differentiate presence of multiple compounds from the toxicological contribution of each compound. Polypharmacy is a key element of cases to be interpreted on the basis of reviews of adverse events at the NPS.

The effect an individual substance can have can be greatly different if it has a pharmacodynamic interaction. Combination of two compounds with depressant properties can cause increased respiratory or central nervous system depression and combinations of two compounds with

stimulant properties can cause increased cardiovascular and neurological stress. NPS may have pharmacological properties that are different from those of conventional drugs and therefore interaction may be unpredictable. When there is limited information on the NPS, data from a known drug cannot be used to automatically infer information on the NPS.

Another complication is pharmacokinetic interactions. The absorption, metabolism or elimination of one material may be affected by another, which may change the concentration or duration of the pharmacologic effects. These interactions may be especially challenging to recreate after death due to the influence of postmortem redistribution and decomposition. As a result, the exposure profile of the measured concentration does not necessarily represent the that actually occurred during life (Ferner 2008).

The individual susceptibility and tolerance should be taken into account as well. Although there are similarities in the effects of a certain concentration, it can be different in another person, depending on the individual's prior exposure, tolerance, health status, genetic makeup, and concurrent medications. This is particularly challenging for NPS in establishing universal concentration thresholds. Analytical results of published forensic toxicology reviews are thus best interpreted in the broader clinical, pathological and circumstantial context.

Another significant issue is attribution. It may not be appropriate to identify several substances as the sole cause of death if only one is identified, without supporting evidence. Rather, the evidence can lead to a conclusion that the NPS caused a synergistic effect. The distinction becomes more significant if the detection of a conventional substance with a known toxicity is found in conjunction with an emerging NPS. The interpretation should, therefore, take into account pharmacological plausibility, relative toxicological significance, concentration patterns and compatibility of the results with the known mechanism of action of each substance.

The literature thus suggests a multi-dimensional approach to polydrug cases. The comprehensive nature of the substance profile should be taken into consideration by toxicologists rather than viewing each analyte individually. This way, the death isn't

attributed to an NPS merely because it's a novel analysis or because it is a death to which the analysis is targeted. It also acknowledges the possibility that NPS can be part of a larger trend of multi-toxin drug poisoning.

6. Conclusion

Novel psychoactive substances (NPS) are a constant and dynamic problem in PMT, as they have undergone rapid structural changes, a variety of pharmacological effects, and the gap between their emergence in the market and validated analytical methods. This is made difficult by limited reference standards, extensive metabolism, matrix effects and the failure of conventional immunoassays and the very limited, narrowly targeted screening assays, requiring integrated use of LC–MS/MS, high-resolution mass spectrometry and complementary targeted, suspect and non-targeted screening strategies. When identified, interpretation is complicated by postmortem redistribution, instability of analytes, the presence of unknown metabolites or different tissue distribution, which can significantly affect measured concentrations as compared to antemortem concentrations. The finding of polydrug combinations makes it even more difficult to attribute toxicity, since synergistic or additive effects on central nervous system, respiratory system or cardiovascular system cannot be reliably derived from concentration data. Therefore, a positive NPS result does not imply causation and requires the toxicological results to be correlated with autopsy, pathological, medical history, circumstances of death and the broader toxicological profile. To maintain analytical readiness, continuous growth of spectral libraries, metabolite characterization, availability of reference materials and sharing information between laboratories are needed. In the end, the most reliable medicolegal determinations will be made through flexible, high resolution analytical methods, and with careful interpretation keeping in mind the uncertainty of potency, redistribution, and inadequate toxicological data for so many emerging compounds. Forensic toxicology can only contribute to the determination of cause and mechanism of death in NPS cases in such an integrated approach.

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