European Monitoring Centre
for Drugs and Drug Addiction

TECHNICAL REPORT
An analysis of post-mortem toxicology practices in drug-related death cases in Europe

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Legal notice

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Background and objectives

In the past decade, between 7 000 and 9 000 drug-related deaths (DRDs) have been reported in Europe every year (EMCDDA, 2018). Most are classified as such on the basis of toxicological investigations (more than 85 % of overdose deaths in the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) Statistical Bulletin are reported with 'known toxicology'). However, only fragmentary information is given concerning the limitations of the data, such as regional or national differences in the analytical capacity of forensic toxicology laboratories. The objective of this report is to provide an updated analysis of the post-mortem toxicology practices of DRD cases in Europe and to discuss the effect of these practices on the monitoring of DRDs. There were two components to this project: a scoping study and a mapping survey.

The **scoping study** analyses the international and national guidance relating to the post-mortem investigation of suspected DRD cases. Criteria for analysis included minimum requirements, recommendations regarding sampling, processing, confirmatory testing, 'general unknown screening', technical specifications and reporting with special reference to new psychoactive substances (NPS).

The **mapping survey** was conducted from May to August 2017, in which 54 forensic toxicology laboratories from 27 European Union (EU) Member States, plus Norway, Turkey and Switzerland, were asked about their technical equipment, analytical strategies and standards for post-mortem investigations, their technical coverage of typical drugs of abuse with special reference to NPS, their reporting standards and potential hindrances to their daily work.

Key findings

The scoping study found that at the European level, but also at the broader international level, there are no specific up-to-date guidelines on forensic toxicology investigations for DRDs, except for single substance groups such as fentanyl and its analogues. General forensic toxicology guidelines follow international accreditation standards. While these are applicable to post-mortems, which follow the same general principles of quality assurance, there are some specific aspects in the case of drug poisoning that are not covered, such as the collection of samples other than blood and urine. The guidelines are also generally quite limited in their statements on minimum standards. It is also important to note that screening for NPS in post-mortem specimens requires up-to-date technical equipment, and therefore it is generally limited to specialised laboratories. Non-targeted comprehensive/'general unknown' screening (GUS) methods are included in current international guidelines but are not generally recommended as a minimum requirement in post-mortem investigations. The survey demonstrated that targeted screening with second-step confirmation is still a relevant approach. The guidelines indicated some differences across countries with regard to the practices and, therefore, to the sensitivity of toxicological investigations. This affects the comparability of the available data and should be reflected in the analysis and presentation of the data on DRDs.

The mapping survey studied 54 laboratories in 30 countries (27 EU Member States, Norway, Turkey and Switzerland) and found that, in 11 EU Member States and in Turkey, all or the majority of the national DRD-associated toxicological investigations are processed centrally in a single laboratory. During the past 5-10 years, the majority of laboratories in Europe have changed the most common combination of techniques used for the detection of drugs or drug metabolites. Instead of immunoassay, followed by confirmation of presumptive-positive specimens by gas chromatography-mass spectrometry, laboratories are switching to multi-target methods using high-tech equipment, for example liquid chromatography-tandem mass spectrometry, high performance liquid chromatography time-of-flight mass spectrometry or ultrahigh-performance liquid chromatography high-resolution high-accuracy mass spectrometry. A change from one method or standard to another will influence trends.

Two thirds (68 %) of participating laboratories are equipped with advanced technology allowing them to undertake comprehensive screenings in the case of poisoning deaths. Among those laboratories with limited technical equipment for some analytes, 47 % reported being able to send biological samples to a specialised laboratory.

As would be expected, laboratories with advanced technical equipment are able to detect a greater range of substance groups. The drugs of abuse most related to deaths, such as opiates, cocaine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA) and methamphetamine, are able to be determined by nearly 100 % of forensic laboratories. However, only around 75 % of the laboratories include tests for buprenorphine, fentanyl or the antiepileptic pregabalin in their routine analysis. Among the NPS, synthetic cathinones (82 %) and phenethylamines (71 %) were most commonly tested for, followed by synthetic cannabinoids and piperazines. In addition to forensic toxicology testing, the exchange of case-related information between laboratories and institutions such as the police, hospitals and forensic pathologists is necessary for the proper interpretation of findings, and one third of the participating laboratories were unsatisfied ('very unsatisfied', 21 %, and 'not satisfied', 13 %) with the extent to which this currently occurs.

Limitations

The representativeness of the survey results was questionable in some large EU Member States, where post-mortem analyses are distributed across many laboratories. The private sector was not involved in the study; however, in most countries it is unlikely that many private laboratories handle the quite specialised and economically less attractive post-mortem analyses. In addition, the survey asked about groups of psychoactive substances, with more specific information obtained for only a small number of single substances.

In general, it was not possible to gain a detailed insight into laboratory processes and to validate ambiguous data, and expert panel recommendations and regulatory instructions by responsible authorities in national languages were not provided systematically. Finally, the study could not reveal distinct timelines for the past 20 years in terms of the development and implementation of new methods and new devices for individual laboratories.

Conclusions

At the interface between national regulations for inquests into cause-of-death investigations and guidelines for toxicological examinations, it would be beneficial to have specific medico-legal recommendations for decision-making on ordering toxicological examinations following autopsies. They should include guidance for cases in which findings at autopsy suggest any ambiguity over cause of death.

There remains an urgent need to increase the screening capabilities of many toxicology laboratories. In countries with a decentralised organisation of forensic laboratories, establishing national reference laboratories to determine certain NPS groups in biological samples could be a strategic option. National or international guidelines on analytical laboratory standards need to be updated to take account of new developments in multi-targeted toxicological analysis.

Although rarely investigated, the capacity of an analytical laboratory is an important factor to consider when interpreting the role of different substances in DRDs. Laboratory capacity has gained even more importance given the rise in NPS and the high prevalence of fatal polydrug poisonings.

Importantly, over time, with the technical developments in terms of laboratory capacity, cases that would have been missed before can be identified. This improvement in detection should be kept in mind and documented when analysing data on direct or indirect DRDs associated with certain

substance groups, such as prescription opioids and NPS. Indeed, some of the observed increase might reflect the improved detection of cases that would have otherwise remained unnoticed. National longitudinal analyses could be used for a comparative analysis between countries with similar incidences of newly occurring substances. Such comparisons are particularly promising in multi-city studies or in comparisons between smaller countries with clearly assigned regional responsibilities for forensic laboratories. Analysis of DRDs will be enhanced by including toxicological laboratories — where this is not yet the case — more sustainably in information networks, for example through the Reitox national focal points.

The technical capacity of toxicological laboratories has already been adjusted to meet the new requirements in many countries. However, new analytical methods inevitably lag behind the first appearance of an NPS. The exchange of mass spectrometry libraries of new analytes and of reference standards between institutions is promoted by the European information system and the European Database on New Drugs (EDND). There is an urgent need to promote the use of existing databases (such as the EDND) and accelerate the exchange of information between specialised national (reference) laboratories on NPS in order to increase the speed of analytical development.

Introduction

High rates of drug-related deaths (DRDs) are a key area of concern for drug policy across Europe. However, monitoring these deaths, identifying actions to reduce them and evaluating their impact are hampered by concerns about the reliability and comparability of data on DRDs. For example, diversity in the national country-level structures and processes are likely to have an impact on the comparability of DRD data across Europe. On the one hand, the number of registered DRDs depends on the prevalence of actual overdose deaths, which is influenced by different regional, national and international triggers, such as the number of problem drug users and diverse risk factors for those who are at risk. On the other hand, changes in the detection rate of actual deaths can influence the number of reported DRDs. This report investigates the variability in guidance, standards and practice in post-mortem toxicological investigation of DRDs across the European Union (EU) in order to provide insights into the extent to which these may influence reported trends. This will help those tasked with interpreting the data to inform policymaking and those tasked with improving the structures and processes in place for the investigation, recording of and reporting of DRDs.

The recognition of a death as drug related depends on the organisation of the post-mortem examination at the scene of death, which varies across countries and can — depending on competence — be considerably influenced by legal regulations and decisions by the police as well as by the judiciary or the health sector. A detailed investigation of a fatality may produce different results depending on the technical resources used. These resources relate to the methods and procedures for obtaining findings from the corpse by autopsy or, more recently, imaging techniques for obtaining information on items or samples collected at a scene of death but also for including information from the social environment, relatives or treating physicians.

The quality of the investigation of a suspected death, however, is greatly affected by the quality of toxicological investigations of biological materials. It is known that, in the case of suspected DRDs, the choice of toxicological investigations is not obvious. Even if an autopsy is performed and the macroscopic and histological results do not definitively clarify the cause of death, it is often the case that a separate decision needs to be made by the contracting authority about whether or not a toxicological examination should take place. Even if toxicological analyses are ordered, differences in the analytical strategy between laboratories can lead to different results owing to variation in the substances tested for, as well as to different limits of detection (LODs). The technical equipment of a laboratory, the competence of the personnel, the financial resources for the maintenance and quality assurance of the results, and the development of methods for the analysis of new psychoactive substances are all important influencing factors on toxicology results.

Such differences may influence statistics on the number of registered DRDs and may lead to differences between countries in the ability to detect new causes of poisoning deaths. Last but not least, a toxicological analysis can contribute to the epidemiologically reported prevalence only if it is integrated into the results of the post-mortem examination. The ultimate cause of death then needs to be reported in a timely manner to the General Mortality Registries or Special Registers. Finally, the rules for the interpretation of toxicological results and their coding should be consistent, and they should conform to the relevant national and European (EMCDDA) definition of a DRD.

The objective of harmonising the registration of DRDs in EU Member States has been accompanied in the past 20 years by numerous analyses of the various factors influencing the process of registering cases at national level. However, relatively little is known about the extent of regional or national differences in the performance of toxicological analyses. Those differences did not appear to be a relevant factor for established drugs of misuse, such as heroin or 'established' opiates. However, the issue remains because of the increased prevalence of use of some prescription opioids and the analytical challenges relating to the constantly evolving NPS on the market, some of which may contribute directly or indirectly to DRDs.

The first section of this report describes a scoping study (Part 1), which analyses the international and national guidance with regard to toxicological post-mortem investigations of suspected DRD cases. This includes minimum requirements, recommendations by national expert associations regarding sampling, processing, confirmatory testing, general unknown screening (GUS), technical specifications and reporting with special reference to NPS.

In the second section, the findings of a mapping survey of the typical or standard toxicology practices in place in each EU Member State and in Norway and Turkey are presented (Part 2). Experts on toxicological analysis based in laboratories undertaking post-mortem analyses were identified and asked to participate in a survey on laboratory performance in suspected DRD cases, guidelines and standards for laboratory practice and reporting, analytical strategies, technical equipment, and potential hindrances/challenges to their daily work on DRD cases.

Finally, based on these results, some general conclusions are drawn and the potential implications are discussed with regard to how to interpret drug-induced deaths prevalence data, taking into consideration the background of toxicology standards and capacities in different countries.

Part 1: Scoping study on national reference documents that address drug-related death toxicology investigations in Europe (28 EU Member States plus Norway and Turkey)

1.1. Objective and methods

The scoping study analyses the international and national guidance on the post-mortem investigation of suspected DRD cases. It includes guidelines published by international and national professional associations or national toxicologist working groups, as well as documents published by national authorities or international organisations regarding scientific or expert recommendations. The evaluation of these documents took account of decision-making about post-mortem forensic toxicological investigations, minimum requirements regarding the sampling and storage of post-mortem specimens, the processing of samples, technical equipment, standards for screening and confirmatory testing with special reference to NPS, interpretation and reporting (prioritising polydrug poisonings). Implications for the monitoring of DRDs at a European level are briefly discussed and areas for improvement are suggested.

Search strategies for relevant documents were carried out between April and August 2017 and included:

- Sources: PubMed, TOXNET, Web of Science
- Searching mode with the following filters:
    o ('forensic toxicology'[MeSH Terms] OR forensic toxicology[Text Word]) AND (('autopsy'[MeSH Terms] OR 'autopsy'[All Fields] OR 'post-mortem'[All Fields]) OR post-mortem[All Fields]) AND ('standards'[Subheading] OR 'standards'[All Fields] OR 'reference standards'[MeSH Terms] OR ('reference'[All Fields] AND 'standards'[All Fields]) OR 'reference standards'[All Fields]) AND drug[All Fields]
    o ('forensic toxicology'[MeSH Terms] OR forensic toxicology[Text Word]) AND standards AND drug OR 'drugs of abuse' AND death OR poisoning
    o ('forensic toxicology'[MeSH Terms] OR forensic toxicology[Text Word]) AND standards AND drugs OR drugs of abuse AND death OR poisoning
- A Google browser-based search: 'Forensic toxicology' AND (guidelines OR standards) AND drugs OR 'drugs of abuse' OR 'illegal drugs' OR 'controlled substances' OR 'new psychoactive substances' OR 'legal highs' OR drug- related AND death OR poisoning
- A targeted document search on the following websites: International Association of Forensic Toxicologist (TIAFT), United Nations Office on Drug and Crime (UNODC), American Association of Forensic Toxicologist (AAFS), Scientific Working Group for Forensic Toxicology (SWGFTOX), Nordic Association of Forensic Toxicologists, Society of Forensic Toxicologists (SOFT), US Food and Drug Administration (FDA), European Medicines Agency and International Union of Pure and Applied Chemistry
- Personal communication with relevant experts taking part in the survey (Part 2).

The following components were explored:

- decision-making on post-mortem forensic toxicology investigations;
- pre-analytical management;
- sample preparation;
- calibration;
- standards for post-mortem screening and confirmatory analytical methods;
- analytical methods for NPS;
- interpretation and reporting of findings;
- toxicological reporting and certification.

1.2. Findings

1.2.1. Coverage of post-mortem toxicology in national and international guidelines

Box 1 **Forensic toxicology definitions**

Toxicology involves the analysis of how chemical substances affect living organisms. The most common applications are workplace drug testing, doping control in sport and human performance toxicology (detecting the presence or absence of drugs or alcohol in the human body, which may be necessary in impaired driving, road traffic accident and sexual assault cases).

Forensic toxicology is concerned with cases in which adverse effects of chemical substances could have administrative or medico-legal consequences. Standards for toxicological DRD investigation fall into the field of post-mortem forensic toxicology (death investigation toxicology), defined as a methodology for the determination of drugs and their metabolites, chemicals such as ethanol and other volatile substances, carbon monoxide and other gases, metals and other toxic chemicals in human fluids and tissues, and for evaluating their role as a determinant or contributory factor in the cause and manner of death.

The toxicology report provides key information to a pathologist, who considers it in the context of the findings of medical conditions at autopsy and the investigative history of a case.

Toxicology results from biological samples are examined in many countries by medico-legal institutions. Seized drugs are typically analysed in police laboratories.

In line with international accreditation standard ISO/IEC 17025 (General requirements for the competence of testing and calibration laboratories), there are a number of guidance documents that represent *all* fields of forensic toxicology. They include requirements for laboratory staff responsibilities, running of a quality management system, method calibration and validation, specimen collection, labelling and handling including security, and chain-of-custody of specimens. External proficiency programmes monitor both the assay and the staff performing the work (United Nations International Drug Control Programme, 1995, 1997, 1999; SOFT/AAFS, 2006; GTFCh, 2009; Cooper et al., 2010; Drummer, 2010).

These guidelines are fully applicable to post-mortem investigations, which follow the same general principles of quality assurance and methods and involve validation according to standard parameters: selectivity, calibration model, stability, accuracy, precision, lower limits of quantification (LOQs), LODs, recovery, reproducibility and robustness (Peters et al., 2007; Scientific Working Group for Forensic Toxicology, 2013).

Specimen collection at autopsy, the potential impact of post-mortem matrix-related effects on standard analytical methods and the interpretation of recommendations are examples of issues relating to post-mortem investigations that need to be addressed specifically in some documents. With regard to analytical methods, guidelines addressing systematic toxicological analysis (STA) involving GUS are particularly relevant to post-mortem toxicology (where the confirmation or exclusion of expected substances is of minor importance) and have increased in importance as markets for increasingly diverse NPS are steadily growing (EMCDDA and Europol, 2016; Guillou, 2017).

In total, 17 references were identified, from 8 countries and 6 international institutions, dating from 1995 to 2017 (Table 1). There were specific recommendations for post-mortem toxicology (and, therefore, indirectly applicable to DRDs) in 12 out of 17 references identified.

TABLE 1
Relevant international and national guidance/reference documents

No	Country/organisation (alphabetical order)	Author/editor	Title (reference)	Last edition	Scope: post-mortem applications (PA)/general recommendations (GR)
1	Czechia	Czech Society for Legal Medicine and Forensic Toxicology	(in Czech) Metodický pokyn pro postup p i toxikologickém vyšet ení speci kovaných návykových látek v krvi a nebo v mo i. [Methodical guidance for the process of toxicological testing of specified substances of the substance or in the blood] Czech Society for Legal Medicine and Forensic Toxicology, expert personal communication 2012)	2012	GR: analysis, interpretation, quality assurance, documentation
2	France	Société Française de Toxicologie Analytique (SFTA)	Recommandations pour la réalisation des analyses toxicologiques dans les cas de décès impliquant des NPS (SFTA, 2017)	2017	PA: sample collection, analysis, minimum list of NPS to be included
3	Germany	Gesellschaft für Toxikologische und Forensische Chemie Arbeitskreis Qualitätssicherung (GTFCh) [Society for Toxicological and Forensic Chemistry Working Group Quality Assurance]	(in German) Anhang D zur Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen Empfehlungen zur Asservierung von Obduktionsmaterial für forensisch-toxikologische Untersuchungen und spezielle Aspekte der Post-mortem-Analytik (attachment to 2) (GTFCh, 2004)	Version 1 2004	PA: sample collection GR: container type, labelling, logging, shipping, storage (no specific DRD recommendations)
4	Germany	GTFCh	(in German) Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen (GTFCh, 2009)	Version 1 2009	PA: analysis (standard addition method) GR: analysis, quality assurance and control, reporting, interpretation (no specific DRD recommendations)
5	Germany	GTFCh	(in German) Anhang A zur Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen Qualitätsanforderungen an die Bestimmung spezieller Analyten aus biologischen Matrices	Version 1 2009	GR: analysis of amphetamines, methamphetamines, methylendioxyamphetamines, cocaine, benzoylecgonine, opiates/opioids. LOD, LOQ for drugs of abuse (no specific DRD recommendations)

No	Country/organisation (alphabetical order)	Author/editor	Title (reference)	Last edition	Scope: post-mortem applications (PA)/general recommendations (GR)
			mit Tabellenanhang (aktuelle Vorgaben zu Bestimmungsgrenzen) (attachment to 2) (GTFCh, 2009)		
6	Poland	Polish Society of Forensic Medicine and Criminology	(in Polish) Zalecenia w sprawie pobierania materialu sekcyjnego do badań toksykologcznych [On the collection of autopsy material for toxicological investigations] (Polish Society of Forensic Medicine and Criminology, 2017)	2012	PA: sample collection
7	Spain	Generalitat de Catalunya/Departament de Justícia/Institut de Medicina Legal de Catalunya	Specific recommendations for the unification of judicial autopsies (Institut de Medicina Legal de Catalunya, 2013)	2013	PA: sample collection/recommended amounts
8	United Kingdom	UK and Ireland Association of Forensic Toxicologists	Forensic toxicology laboratory guidelines (Cooper et al., 2010)	2010	PA: sample collection/recommended amounts, post-mortem and sample changes; external proficiency testing programme; analysis of alcohol, review of data before reporting, GR: sample collection, container type, labelling, chain-of-custody, logging, transport, storage, analysis, quality assurance and control, reporting, interpretation
9	Switzerland	Work Group on Drugs of Abuse Testing (SCDAT/AGSA) Swiss Association of Pharmacists (pharmaSuisse) • Swiss Society of Clinical Chemistry • Swiss Society of Legal Medicine • Swiss Association of the Diagnostic Equipment and Product Industry • University of Bern	Guidelines for drugs of abuse testing (SCDAT/AGSA, 2012)	2012	PA: sampling/specimen to be taken; technical recommendations for quantitative analysis of substance groups GR: analysis, interpretation, quality assurance, documentation ((no specific DRD recommendations)

No	Country/organisation (alphabetical order)	Author/editor	Title (reference)	Last edition	Scope: post-mortem applications (PA)/general recommendations (GR)
10	USA	Laboratory Guidelines Committee of the Society of Forensic Toxicologists (SOFT) and the Toxicology Section of the American Academy of Forensic Sciences (AAFS)	Forensic toxicology laboratory guidelines (SOFT/AAFS, 2006)	2006	PA: sample collection GR: sample collection, container type, labelling, chain-of-custody, logging, transport, storage, analysis, quality assurance and control, reporting, interpretation
11	Council of Europe	Committee of Ministers to Member States	Recommendation no R (99) 3 on the harmonization of medico-legal autopsy rules (Committee of Ministers to Member States, 2000)	1999	PA: sample collection
12	International Association of Forensic Toxicologists	TIAFT Committee of Systematic Toxicological Analysis	Recommendations on sample preparation of biological specimens for systematic toxicological analysis (Stimpfl et al., 2011)	2011	PA: sample collection GR: sample pre-analytic treatment, sample extraction methods
13	International Association of Forensic Toxicologists	Scientific Working Group for Forensic Toxicology (SWGTOX)	Standard practices for method validation in forensic toxicology (Scientific Working Group for Forensic Toxicology, 2013)	2013	GR: method validation
14	United Nations International Drug Control Programme (UNDCP)	UNDCP Scientific Section	Recommended methods for detection and assay of heroin, cannabinoids, cocaine, amphetamines, methamphetamine and ring-substituted amphetamines in biological specimen (UNDCP, 1995)	1995	GR: sample collection, container type, labelling, chain-of-custody, logging, transport, storage, analysis, quality assurance and control, reporting, interpretation
15	UNDCP	UNDCP Scientific Section	Recommended methods for detection and assay of barbiturates and benzodiazepines in biological specimen (UNDCP, 1997)	1997	GR: sample collection, container type, labelling, chain-of-custody, logging, transport, storage, analysis, quality assurance and control, reporting, interpretation
16	UNDCP	UNDCP Scientific Section	Recommended methods for detection and assay of lysergide, phencyclidine, psilocybin, methaqualon in biological specimen (UNDCP, 1999)	1999	GR: sample collection, container type, labelling, chain-of-custody, logging, transport, storage, analysis, quality assurance and control, reporting, interpretation
17	UNODC	UNODC	Recommended methods for the identification and analysis of fentanyl and its analogues in biological specimens	2017	Specific to fentanyls: PA: sample collection, post-mortem and sample changes, interpretation GR: sample collection,

No	Country/organisation (alphabetical order)	Author/editor	Title (reference)	Last edition	Scope: post-mortem applications (PA)/general recommendations (GR)
			(UNODC, 2017)		stabiliser, storage, screening and confirmation methods

1.2.2. Decision-making on the application of post-mortem forensic toxicological investigations in suspected drug-related deaths

There are no generally accepted principles for decision-making regarding the ordering of toxicological examinations after autopsies in EU countries. Such guidelines could be used at the interface of the jurisdiction of the police, legal medicine and prosecutor. Recent US recommendations specify inclusion criteria for the toxicological analysis for controlled substances if one or more of the following apply to a case (Davis, 2014):

- known history of prescription opioid or illicit drug use, misuse or abuse;
- evidence of opioid or illicit drug abuse revealed by scene investigation;
- autopsy findings suggesting a history of illicit drug abuse;
- massive lung oedema and froth in airways present with no grossly visible explanation (e.g. heart disease) or other non-toxicological explanation (e.g. epileptic seizure) (Dinis-Oliveira et al., 2012);
- potential or suspected smugglers of illicit drugs (mules);
- no unequivocal cause for death identified at autopsy;
- decedents with a potential natural cause of death visible at autopsy whenever a drug may have precipitated or contributed to death by an additive mechanism, such as opioid-induced respiratory depression;
- traumatic deaths.

There are currently no comparable specific recommendations from EU Member States available. Guidelines for toxicological investigations have their first point of application in principle during the first pre-analytical measures, but they do not refer to whether case-related toxicological investigations should be initiated at all. The decision here depends on the recommendation of forensic pathologists after autopsy but apparently follows a general algorithm for the clarification of the cause of death rather than DRD-specific considerations.

1.2.3. Pre-analytical management: sample collection, preservatives, storage of post-mortem specimens, preparation and extraction

Box 2 describes the basic procedures in forensic toxicological analyses. These have an impact on the ability of these analyses to contribute to the identification of DRDs and include pre-analytical management as well as the analytical procedures themselves. In general, urine and blood are the most frequently used liquid specimens in post-mortem analysis. Urine is less sensitive in the event of rapid death after drug use (Stimpfl et al., 2011).

Most guidelines do not make specific recommendations as regards the types of specimen to be collected in DRD cases. The general SOFT/AAFS (2006) guidelines suggested heart blood, peripheral blood, bile, urine, gastric contents, liver, kidney and brain for routine sample collection without prioritisation. German guidelines recommend peripheral and cardiac blood, stomach contents and urine as basic specimens, supplemented by hair, bile, liver, lung, brain and kidney in all cases of unclear cause of death at autopsy. UK/Irish guidelines suggest, in accordance with TIAFT guidelines for post-mortem collection in general, the routine sampling of peripheral blood and urine, and the sampling of 'heart blood, peripheral blood, bile, urine, gastric contents, liver, kidney, brain and hair' only after consultation with the laboratory. Lung and intestine specimens may be needed for unique

poisons (not specified) (Cooper et al., 2010, Stimpfl et al., 2011). Recent US recommendations specify blood, urine and vitreous humour as minimum standard autopsy specimens for toxicological analysis in suspected opiate-related deaths (Davis, 2014).There are no DRD specifications regarding collection in the event of severe decomposition or skeletisation.

Specifically in relation to DRDs, the European Council guidelines from 1999 (published in 2000) recommended that vitreous humour, brain tissue, injection marks and hair should be collected in addition to a basic standard sampling (Committee of Ministers to Member States, 2000). Deaths relating to opioids should include the sampling of blood, vitreous humour, urine, bile and gastric contents (Davis, 2014). Other tissue samples considered but not included in general recommendations are brain stem/cerebellum segments in relation to opiate-/opioid- and cocaine-related deaths, which may reflect drug concentrations at their site of action (Stimpfl and Reichel, 2007). Pericardial fluid was also proposed for cocaine-related deaths (Contreras et al., 2006; Contreras et al., 2007) but has not come into widespread use.

With regard to the quantities of each specimen to be collected, guidelines specify quite consistently 10 ml as the minimum amount for peripheral blood and whatever amounts are available for urine and vitreous humour (Cooper et al., 2010; Institut de Medicina Legal de Catalunya, 2013). Blood from the femoral vein is preferred over blood from other sites such as the subclavian vein, right atrium or any intact blood vessel (Dinis-Oliveira et al., 2010, Polish Society of Forensic Medicine and Criminology, 2012; Davis, 2014). Peripheral blood should be collected in two different tubes with no air pockets and at least one of them should include sodium fluoride as a preservative (GTFCh, 2009) and potassium oxalate as an anticoagulant. Bile is recommended for its significance as an alternative route of elimination (Institut de Medicina Legal de Catalunya, 2013). Hair should be collected from the head or the armpit or pubic areas if this is not possible, primarily for storage and analysis in the event of positive blood and urine/bile results (Institut de Medicina Legal de Catalunya, 2013).

Box 2 **Basic procedures in forensic toxicological analyses**

Sample collection: urine and blood are the most frequently used liquid specimens from the human body but the condition of a corpse may require the collection of different liquids or tissues.

Storage conditions: post-mortem metabolism needs to be controlled if possible, otherwise the active metabolites of drugs may be lost.

Sample preparation: conventional approaches require the separation of a substance from its accompanying matrix ([1]). As there is no single extraction procedure for STA covering all relevant substances, complementary techniques have to be combined (Stimpfl et al., 2011). A full 'clean-up' of a sample is impossible; components from a sample matrix will be co-extracted. However, in laboratories with the latest technology (see Box 3), substances are identified through their elemental composition, so matrix components can be identified.

Method calibration: the comparison of measurement values delivered by a device under test with those of a calibration standard of known accuracy. An adequate matrix for calibration is a challenge in post-mortem toxicology. If analyses are performed on unusual specimens (decomposed tissue, vitreous humour, etc.), appropriate matrix-matched calibrators should, when possible, be prepared and tested concurrently with the specimen (SOFT/AAFS, 2006).

Accuracy, validity and reliability: this needs to be proved for each method before samples are measured in order to minimise any uncertainties in the measurement.

([1]) In chemical analysis, 'matrix' refers to the components of a sample other than the analyte of interest. The matrix can have a considerable effect on the way the analysis is conducted and the quality of the results obtained; such effects are called matrix effects.

Blood sampling before opening the body minimises the risk of sample contamination. The site, time and date of blood sampling should always be recorded (SOFT/AAFS, 2006; Flanagan, 2012-2013). In

the case of prolonged survival, blood samples from the date of hospital admission should be secured as soon as possible (GTFCh, 2009). Short-term storage conditions for specimens and the use of anticoagulants/preservatives for the stabilisation of liquids against *in vitro* metabolism must be defined.

Recommendations for sample pre-treatment specify the following: sample hydrolysis, precipitation in the case of high protein contents and homogenisation (necessary for nearly all post-mortem specimens), followed by extraction (liquid-liquid extraction or solid-phase extraction), purification, concentration and derivatisation (if gas chromatography mass spectrometry (GC-MS) is applied) (UNDCP, 1995; Stimpfl et al., 2011).

1.2.4 Calibration in the case of post-mortem specimens

There are major challenges regarding the analysis of post-mortem specimens compared with the routine analysis of body fluids in living humans (e.g. linked to matrix effects) (Staeheli et al., 2015).

In post-mortem analyses, a directly comparable reference matrix is often not available, so validation of these parameters needs individual solutions. In such cases, guidelines (SOFT/AAFS, 2006; GTFCh, 2009) recommend the standard addition method (SAM), which allows for semi-quantitative determination, in which the calibration function is generated directly in the sample matrix. This approach takes into consideration the matrix properties of that specific case (including post-mortem changes). In this method, the sample to be examined is processed and measured in a completely identical manner, first unchanged and then again after the addition of defined amounts of the active compound to be determined. The concentration of the compound to be added should correspond to the highest expected sample concentration. If the sample quantity is sufficient, several different concentrations should be added. The original analyte concentration in the sample can then be deduced by linear regression.

1.2.5. Standards for post-mortem screening and confirmatory analytical methods

Before 2010 (but still standard practice in many laboratories), analytical techniques relied heavily on immunoassay screening analyses and mass spectrometry (MS) for confirmatory analyses using either high-performance liquid chromatography or gas chromatography as the separation technique (SOFT/AAFS, 2006; Contreras et al., 2006). A Substance Abuse and Mental Health Services Administration guidelines consensus panel on 'Uniform standards and case definitions for classifying opioid-related deaths' noted in 2013 that 'the most common combination of techniques used for the detection of drugs or drug metabolites in urine is immunoassay, followed by confirmation of presumptive-positive specimens by gas chromatography-mass spectrometry'(Goldberger et al., 2013).

Essentially, there are no major differences between post-mortem forensic toxicology (including DRD analysis) and other applications (e.g. human performance forensic toxicology or forensic drug testing in live humans) with regard to the quality assurance of analytical strategies and quality control (e.g. acceptable deviations of control results, use of deuterated internal standards for MS, ionisation methods, interpretation of mass spectra using a minimum number of qualifying ions for each analyte in MS).

Box 3 **Technological changes in forensic laboratories**

Available equipment and techniques

Over the past 10-15 years, conventional techniques such as GC-MS have been supplemented by more advanced technology. GC-MS has been a widely used methodology for limited GUS but the application is restricted to substances suitable for gas chromatography and electron or chemical ionisation, where appropriate after derivatisation (Meyer et al., 2010).

Liquid chromatography-tandem mass spectrometry (LC-MS-MS) expands significantly the coverage of substances, exploiting the polarity and low volatility of many new substances and metabolites. Liquid chromatography allows more substances to be analysed in even lower concentrations on liquid rather than gaseous carrier-facilitated separation. It relies on pumps to pass a pressurised liquid solvent containing the sample mixture through a column filled with a solid adsorbent material.

High-performance liquid chromatography time-of-flight mass spectrometry (HPLC-TOF-MS) allows determination of substances directly through accurate mass. It has become a method of choice for a comprehensive screening.

Ultrahigh-performance liquid chromatography (UHPLC), combined with TOF-MS allows additional selectivity, sensitivity and speed resulting from increased chromatographic resolution. Both molecular weight information and structural details of unknown analytes are gained by high-resolution high-accuracy mass spectrometry (HRMS) enabling accurate-mass determination of ionic species obtained from drugs and their metabolites. HRMS and Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS) allow measurements at extraordinarily high resolution.

Accurate-mass databases and spectral libraries are used for peak identification, which contain accurate mass collision-induced dissociation spectra of more than 2 500, and theoretically calculated accurate mass data of several thousands of toxicologically relevant substances (Maurer et al., 2016; Noble et al., 2018). Assisted by these techniques, forensic toxicologists can keep up with the detection and identification of both traditional drugs of abuse as well as NPS.

Types of screening: comprehensive screening versus immunological screening

Comprehensive screening means that substances are searched for in a non-targeted manner (GUS). It allows for wide-scope screening of many parent substances, metabolites and transformation products with an acquisition of accurate-mass full spectrum data.

By contrast, the established pre-test strategy of immunological screening is always limited to certain groups of substances, so it will selectively test for what is expected in the sample.

Using technologies such as HPLC-TOF-MS, screening (formerly a preliminary indication of a substance) and confirmation analysis (for secure identification) partially merge together. Methods for preliminary compound identification by liquid chromatography quadrupole time-of-flight mass spectrometry have been proposed when reference standards are unavailable (Tyrkkö et al., 2010; Mollerup et al., 2017).

Quantification

For quantification, however, reference samples are required for 'established' as well as upcoming new substances, regardless of the choice of analytical technique. LOD (the lowest quantity of a substance that can be detected) and LOQ (the lowest concentration that can be quantified) vary considerably depending on various factors, such as the performance of the chosen chromatographic technique and mass resolution.

When immunological and UHPLC-HR-TOF-MS-based screenings have been compared, the latter produce a lower number of false-positive results for the main drug groups (such as cannabinoids, cocaine, opiates, amphetamines) than occur with immunoassay. Many false-negative immunoassay results are a result of higher cut-off concentrations and interference from the matrix, which impede the detection of NPS and prescription drugs (Sundström et al., 2015).

Different extraction and analytical methods are used to monitor different drug classes of interest in biosamples. There is consensus that not all controlled drugs or toxins can be routinely screened or

tested for in death investigations, and so a negative result does not necessarily exclude a particular substance (Byard and Butzbach, 2012). This is a concern particularly when targeted screening is relied on, as these methods would probably fail to detect unknown compounds such as NPS. However, targeted screening is not an outdated concept. Non-targeted GUS methods for arrays of substances are included in current international guidelines (SOFT/AAFS, 2006; GTFCh, 2009) but they are far from being generally recommended as a minimum requirement in post-mortem investigations.

Screening methods

Non-instrumental screening tests (e.g. the analysis of urine at autopsy by test strips) should be regarded as a rough indication for the decision to carry out a further toxicological investigation but should never justify classification of a death as drug related (SCDAT/AGSA, 2012), nor are instrument-based screening tests *alone* adequate for establishing a cause of death in clinical and forensic toxicology (Ceelen et al., 2011; Davis, 2014).

Screening tests must be appropriate and validated for the type of biological specimens being analysed. They may be directed towards a class of drugs, such as opiates, or may be a broad-based screen, such as GC-MS (SOFT/AAFS, 2006) and LC-MS (Cooper et al., 2010). If a reported cut-off point is used (the threshold below which a result is referred to as negative), the precision of the assay around that cut-off point must be demonstrated (SOFT/AAFS, 2006).

In line with these recommendations, the UK guidelines state that in most instances in which a laboratory is asked to look for drugs in biological specimens, screening tests are employed. More recently available techniques that may be available in many laboratories (see above) are not mentioned in the guidelines.

Confirmation methods

It is recommended that the identity of an analyte is confirmed using a different extract of the same specimen as that used for the first test or a second specimen (SOFT/AAFS, 2006). In general, the presence of a substance should be verified in more than one specimen if possible (SOFT/AAFS, 2006).

Use of an immunoassay system based on another screening method to confirm a previous immunoassay is not regarded as acceptable (GTFCh, 2009; Cooper et al., 2010). In addition, the detection of an analyte by immunoassay and 'confirmation' by gas chromatography with propionylation at the N position or gas chromatography FID (flame ionisation detector) does not provide sufficient specificity in forensic toxicology (SOFT/AAFS, 2006; Cooper et al., 2010) and is not recommended.

Before about 2010, some guidelines classified (referring to basic equipment for all areas of forensic toxicology) flame ionisation nitrogen-phosphorus detectors for gas chromatography and diode-array detection (DAD)/ultraviolet/fluorescence detectors for liquid chromatography as alternatives to detection by MS (UNDCP, 1995; GTFCh, 2009). Others recommend MS for confirmatory analysis, 'where possible and practical' (Cooper et al., 2010). In recent guidelines, GC-MS for volatile and heat-stable compounds, as well as HPLC-MS/LC-MS for non-volatile and heat-labile compounds, are recommended methods for the confirmatory quantitative determination of DRD-related substance groups (opiates/opioids, cocaine/metabolites, methadone/metabolites, amphetamines/metabolites, benzodiazepines and 'z-drugs', barbiturates (HPLC-DAD is optional for the last four of these) (SCDAT/AGSA, 2012).

Specific analytical recommendations for opioids

In the United States, a National Association of Medical Examiners position paper from 2014 specifies recommendations for the investigation, diagnosis and certification of deaths related to opioid drugs: a toxicological panel should be comprehensive and include 14 defined opioids, as well as

benzodiazepines and other potent depressant, stimulant and anti-depressant medications (Davis, 2014).

The quantification of codeine and morphine and their major metabolites, particularly morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) is recommended for the assessment of codeine versus morphine- and heroin-related deaths and their survival times, applying codeine to morphine and morphine/M3G/M6G concentration ratios (Schanzle et al., 1999; Berg-Pedersen et al., 2014; Darke and Duflou, 2016). Heroin use is determined by its marker 6-monoacetylmorphine (Darke and Duflou, 2016). For this, multi-analyte LC-MS methods have been reported (Frost et al., 2015; Frost et al., 2016).

Enantioselective analysis for methadone (in combination with the metabolite EDDP) has been proposed for countries with both racemic methadone and enantiomerically pure *R*-methadone available (Jantos and Skopp, 2013; Rodriguez-Rosas et al., 2007; Holm et al., 2012).

Earlier reports on the detection of fentanyl in post-mortem specimens described standardised solid-phase versus liquid-liquid extraction and LC-MS-MS with electrospray source in positive ionisation mode for opioids (Coopman et al., 2007; Teske et al., 2007; UNODC, 2017). Recently published UNODC recommendations describe the potential problem of the lack of sensitivity of immunochemical screening kits of different manufacturers for some fentanyl analogues. GC-MS screening is recommended for the common prescription opioids fentanyl, sufentanil, alfentanil and metabolites, and liquid chromatography high-resolution high-accuracy mass spectrometry (LC-HRMS) is recommended for the screening of a range of opioids/selected fentanyl derivates. GC-MS, LC-MS-MS and ultrahigh-performance liquid chromatography high-resolution high-accuracy mass spectrometry (UHPLC-HRMS) are recommended for confirmatory and quantitative analysis. The isomer differentiation in fentanyl analogues with the same molecular core structure (especially when standard reference materials are not available) is reported to be challenging (UNODC, 2017).

Mass spectrometry-based multi-analyte methods
In recent years, multi-analyte methods have been refined because they allow a much simpler and cheaper identification monitoring of substances of different drug classes in one single body sample (Remane, 2010). Multi-analyte methods (including the application of advanced electrostatic traps) have repeatedly demonstrated their applicability for post-mortem matrices (Dresen et al., 2010; Broecker et al., 2011; Vogliardi et al., 2011; Broecker et al., 2012; Sundström et al., 2013; Montenarh et al., 2015; Maurer et al., 2016). Tandem MS has become preferred over single-stage MS (Sauve et al., 2012). Drug detection in post-mortem human blood by UPLC-MS and UPLC-MS-MS results in higher drug detection rates than GC-MS screening (Rosano et al., 2011) and the LOD in whole blood is lower than the lowest therapeutic concentration listed in blood level lists (Montenarh et al., 2015). Multi-target screening with electrostatic traps is essentially seen as a complementary method to GC-MS screening, HPLC-DAD screening and immunoassays.

However, although 'multi-target' screening can be applied to a huge list of substances, as long as reference standards, as well as analytical methods, are not available, particularly for a majority of NPS and their metabolites, these approaches may still fail to detect some drugs (Favretto et al., 2013).

1.2.6. Analytical methods for new psychoactive substances in post-mortem specimens

A NPS is a new narcotic or psychotropic drug, in pure form or in preparation, that is not controlled by the United Nations drug conventions but which may pose a public health threat comparable to that posed by substances listed in these conventions (EMCDDA definition according to Council Decision 2005/387/JHA) (Council of the European Union, 2005). In the past 3 years, death series have been reported by EMCCDA risk assessment reports for MDMB-CHMINACA, acryloylfentanyl, furanylfentanyl, AB CHMINACA, 5F-MDMBPINACA, 4F-iBF, CUMYL-4CN-BINACA, ADB-

CHMINACA, THF-F, carfentanyl, MT45, aPvP, 4,4'-DMAR and 25I-NBOMe (EMCDDA, 2018). Although the mortality associated with NPS is still not comparable to that of opiate-related deaths, there is uncertainty about the number of undetected cases, and this issue is becoming a more significant challenge for post-mortem forensic toxicology. To clarify the chemical structure of an unknown drug in a 'legal high' seizure, GC-MS, LC-MS-MS, high-resolution MS or TOF-MS can be used (Glicksberg et al., 2016).

Although various human sample matrices are available for testing, urine and blood are the first choices for NPS screening. However, many of these drugs, especially unchanged synthetic cannabinoids, exist in urine and blood for only a short period. Other matrices such as hair and saliva are likely to receive more attention in the future (Namera et al., 2015).

Current literature on the analytical methodologies that can be applied to these samples is still limited and a more thorough validation is often required, including a comparison of the results obtained from conventional approaches and from innovative strategies, in order to determine their suitability (Mercolini and Protti, 2016). Therefore, the absence of national or international recommendations/guidelines — particularly with scope in the post-mortem toxicology field — is not unexpected. In 2017, a list of observed NPS in French DRD casework was published as a reference for compounds to be included in toxicological analysis in suspected cases (Société Française de Toxicologie Analytique, 2017).

A range of literature reports on validated methods for the analysis of a broad spectrum of NPS in urine, whole post-mortem blood, vitreous humour and pericardial fluid (Shanks et al., 2012; Guale et al., 2013; Marinetti and Antonides 2013; Pasin et al., 2015; Sundström et al., 2015, Glicksberg et al., 2016; Margalho et al., 2016; Tynon et al., 2017) (Table 2). Furthermore, the concept of non-targeted screening using different approaches for identification of unknown compounds including software tools for prediction of molecular structures has been proposed as a valuable tool in forensic cases in which intoxication is suspected but no drug is identified by targeted analysis (Mollerup et al., 2017).

TABLE 2
Recently published methods for the analysis of NPS in post-mortem specimens (examples)

Reference	Material	Method	Analyte
Shanks et al., 2012	Post-mortem blood	Liquid-liquid extraction UHPLC-MS-MS in positive electrospray ionisation mode	Synthetic cannabinoids JWH-018 and JWH-073
Guale et al., 2013	Post-mortem blood and urine	LC-TOF-MS after solid-phase extraction	Newer synthetic 'Spice/K2' cannabinoids and cathinone 'bath salt'
Marinetti and Antonides, 2013	Whole post-mortem blood, vitreous humour and pericardial fluid	Liquid-liquid extraction and detection by LC-QTOF-MS	Synthetic cathinones (3,4-methylenedioxypyrovalerone (MDPV), 3,4-methylenedioxymethcathinone (methylone), pyrovalerone, pentylone, alpha-pyrrolidinopentiophenone (alpha-PVP) and methedrone
Pasin et al., 2015	Whole blood	Liquid-liquid extraction followed by LC-QTOF-MS	37 NPS including cathinones, hallucinogenic phenethylamines and piperazines
Glicksberg et al., 2016	Urine and blood	Solid-phase extraction and LC-QTOF-MS	22 synthetic cathinones (methcathinone, ethcathinone, pentedrone, buphedrone, 3-fluoromethcathinone (3-FMC), 4-fluoromethcathinone (4-FMC), 4-methylethcathinone (4-MEC), 4-ethylmethcathinone (4-EMC), mephedrone, methedrone, 3,4-dimethylmethcathinone (3,4-DMMC), ethylone, butylone, pentylone, eutylone, methylone,

			methylenedioxypyrovalerone (MDPV), 4-methylpyrrolidinobutiophenone (MPBP), 3,4-methylenedioxypyrrolidinobutiophenone (MDPBP), α-pyrolidinopentiphenone (α-PVP), pyrovalerone, and naphyrone)
Margalho et al., 2016	Whole post-mortem blood, vitreous humour and pericardial fluid	Mixed-mode solid phase extraction, followed by microwave fast derivatisation and analysis by GC-MS operated in selected ion-monitoring mode	D-cathine (D-norpseudoehedrine), ephedrine, methcathinone, 1-(4-methoxyphenyl)-propan-2-amine (PMA), mephedrone, methedrone, 2,5-dimethoxy-4-methylamphetamine (DOM), 4-bromo-2,5-dimethoxyamphetamine (DOB), 2,5-dimethoxyphenethylamine (2C-H), 4-bromo-2,5-dimethoxyphenethylamine (2C-B), 4-iodo-2,5-dimethoxyphenethylamine (2C-I), 2-[2,5-dimethoxy-4-(ethylthio)phenyl]ethanamine (2C-T-2), 2,5-dimethoxy-4-isopropylthiophenethylamine (2C-T-4) and 2-[2,5-dimethoxy-4-(propylthio)phenyl]ethanamine (2C-T-7),
Tynon et al., 2017	Whole blood	Separate extraction procedures followed by LC-MS-MS	Diverse synthetic cannabinoid drugs, targeting arylindole compounds as well as the emerging aminocarbonyl/carboxamide (NACA) compounds

Note: LC-QTOF-MS, quadrupole time-of-flight liquid chromatography mass spectrometry.

Although strongly recommended, the sharing of reference materials and expertise (e.g. between laboratories in the same country, or between laboratories analysing seized substances or biological samples, or between laboratories in the same international network) is challenging, especially considering the number and rapid evolution of the substances that are detected on the market. The exchange of analytical data is promoted by databases such as the European Database on new drugs (EDND) under the framework of the EMCDDA Early Warning System (EMCDDA, 2007). The detection of use of NPS is a particular analytical challenge where identification of metabolites and/or the parent molecule in biofluids can be difficult because of the low concentrations encountered for the more potent substances and the lack of knowledge about many of them (Guillou, 2017).

1.2.7. Interpretation and reporting of findings in forensic toxicology

The interpretation of concentrations of post-mortem drugs of abuse requires correlation with medical history (prescriptions), scene investigation (drug paraphernalia), seized substances (transdermal patches, pills in or outside vials and autopsy findings) (SOFT/AAFS, 2006; GTFCh, 2009; Davis, 2014).

If death is attributed to any drug or combination of drugs (whether as cause or contributing factor), the certifier should list all the responsible substances by generic name in the autopsy report and on the death certificate (Davis, 2014). In the toxicology report, applied methods should be described, including the substances included, their LOQ and cut-off values (obviously this is not appropriate for reported non-targeted screening results, but international guidance in such cases is missing). No quantitative value from a non-specific immunological or other initial testing procedure should be reported, unless the procedure has been appropriately validated through parallel studies with a reference quantitative method (SOFT/AAFS, 2006).

Consideration needs to be given to potential interactions of pharmacokinetic and pharmacodynamic variables when making a cause of death determination (Davis, 2014). In some cases, a drug can cause death even at a concentration below a reported lethal range. Conversely, the presence of a drug concentration within the reported lethal range does not necessarily make the drug the cause of death. Published tables of therapeutic, toxic and lethal concentrations can be highly misleading (Musshoff et al., 2004; Kennedy, 2010; Launiainen and Ojanpärä, 2013). Tolerance accounts for some part of the overlap between therapeutic and lethal concentrations of opioid analgesics (Ferner, 2008), but there is no reliable quantifiable measure of drug tolerance before or after death (with hair

analysis only providing an indication of tolerance) (Davis, 2014). With regard to NPS, there are often no defined concentration ranges associated with (especially emerging) NPS that would correspond to degrees of toxicity and expected outcomes. However, toxicological significance scores have been proposed that consider a range of different sources of evidence to assign a level of significance (low, medium, high or unclassified) that indicates the likely role of a substance in contributing to or causing death (Elliott et al., 2017).

Box 4 Pitfalls in the interpretation of substance concentrations in post-mortem specimens

Drug concentrations measured in post-mortem samples cannot be used to reliably calculate the precise quantity of medication consumed (Davis, 2014). A drug level can be elevated exclusively because of post-mortem redistribution (exchange between tissues leading to changes in drug blood levels in the post-mortem period) (Cooper et al., 2010). This is particularly relevant for centrally acting drugs with large volumes of distribution (the theoretical volume that would be necessary to contain the total amount of an administered drug at the same concentration that is observed in the blood plasma) (Flanagan, 2012-2013). The interpretation of solid tissue concentrations of drugs is complicated by the fact that drugs may distribute unequally throughout body tissues because of variations in blood flow, bio-accumulation and other factors (Davis, 2014). Reference concentrations in different tissues related to concentrations in post-mortem blood have rarely been reported (Skov et al., 2015). Relevant technical literature should be consulted (Flanagan et al., 2008; Moffat et al., 2011; Suzuki and Watanabe, 2011; Kintz, 2012; Baselt, 2017). Tissue levels may be affected by the post-mortem interval, direct (e.g. stomach) and indirect (bladder diffusion) contamination (GTFCh, 2009), refrigeration before autopsy and the position of the body (Pounder et al., 1996). Endogenous metabolism (e.g. production of gamma-hydroxybutyrate, or GHB) and decomposition involving both autolysis and putrefaction may increase or reduce drug and metabolite levels in post-mortem specimens (e.g. microbial production or loss of alcohol) (GTFCh, 2009; Skopp, 2010) depending on the tissue/organ in question (GTFCh, 2009; Lafreniere and Watterson, 2009; Wyman et al., 2011) and storage conditions (Holmgren et al., 2004). Such effects may be minimised by the prompt refrigeration of the body and by performing the autopsy quickly (Flanagan, 2012-2013).

Conclusions should never be based solely on the drug level detected but should also include an in-depth review of the death scene or clinical scenario and drug characteristics (Patel, 2012); however, interpretation of analytical results is often limited by the inadequate information provided in a particular case (Skopp, 2010).

With regard to deaths involving opiates, the presence of 6-acetylmorphine (6-AM) rather than heroin is sufficient to ascribe intoxication to heroin. It is important that the presence of 6-AM is confirmed by toxicological investigations, as heroin fatalities frequently cannot be distinguished from morphine intoxication by the scene investigation (Gill et al., 2016). In the absence of 6-AM, heroin use can be reasonably inferred by other means (Davis, 2014). A morphine-to-codeine ratio of greater than 1 may be considered evidence of heroin use (Jones and Holmgren, 2011).

1.2.8. Certification of drug-related deaths

In accordance with World Health Organization (WHO) guidelines and death certification legislation, US guidelines recommend that toxicological results should be included on the death certificate only in cases of a pathological contribution to death (Gill and Stajíc, 2012). With regard to the assignment of the cause of death on the death certificate, listing the generic names of all chemical agents considered responsible for causing death is recommended (Department of Health and Human Services, Centers for Disease Control and Prevention, National Centre for Health Statistics, 2003). The recommended approach applies to drugs present in concentrations sufficient to have caused death or contributed to death in a given case. Predisposing physical conditions that might have predisposed the person to a fatal outcome being neither necessary nor sufficient to cause death should be listed in the death certificate as 'other significant conditions' (Davis, 2014).

1.3. Discussion

The scoping study found that although European national or multi-national guidelines identified for forensic toxicology post-mortem investigations — including aspects for DRDs — are in general accordance with the guidelines of international professional societies, there is no agreed guideline from the EU Member States in this field. This results in some differences across countries with regard to the current analytical strategies and practices, which in turn affect the sensitivity of the toxicological investigations of suspected DRD cases. This limits the comparability of the data available, and this should therefore be reflected in the analysis and presentation of the data on DRDs at a European level. The scoping study identifies the various kinds of differences and elements that can influence the results of a toxicology investigation. It provides a description of specific weaknesses and difficulties with regard to the sensitivity and comparability of the data. These are detailed below, along with suggestions for ways forward and recommendations. A high level of specialisation is required for the investigation of post-mortem material from suspected DRD cases and evaluation of the findings. While the complexity of post-mortem analysis is high, the market for these analytical services is relatively limited, compared with markets for highly standardised urine-based doping tests or workplace testing, and post-mortem analyses are frequently restricted to official public bodies and state laboratories.

National expert panel recommendations tend to allow some degree of flexibility, taking account of the fact that minimum standards may put pressure on and be challenging for laboratories with a lack of adequate equipment and/or limited resources for reference standards for constantly emerging new substances appearing on the market.

The absence of specific European guidelines on forensic toxicology for DRD investigations is a barrier to the desired harmonisation of monitoring DRDs. Technical guidelines tend to be limited in their statements on minimum standards (as they are cautious about not excluding laboratories). Guidelines also fast become outdated in times of significant technical advances. It is probable that general technical instructions for the determination of non-opioid NPS are more likely to be developed in the area of human performance toxicology rather than the post-mortem area. Nevertheless, specific expert recommendations at the European level are needed for the inclusion of opioids in analytical strategies, as this is the most important substance group in DRDs.

Furthermore, specific medico-legal recommendations for decision-making on the ordering of toxicological examinations following any type of post-mortem examination would have a promising impact on the harmonisation of DRD monitoring. This should include guidance for cases with an equivocal cause for death identified at autopsy or with a potentially natural cause of death, whenever a drug may have precipitated or contributed to the death by an additive mechanism.

For laboratories specialising in the forensic evidence of NPS, the availability of reference materials (seized substances or reference standards) is essential. This would require the establishment of cooperation between forensic/police laboratories and medico-legal laboratories for the analysis of biological samples, because in many countries these are institutionally separated. Reference standards for the quantitative determination of emerging NPS are often available only — if at all — with a considerable time delay (up to years) from the relevant industrial suppliers. However, they are produced under rigorous quality assurance standards. The use of confiscated samples for reference purposes may be a compromise. However, it requires information exchange on where and when high-purity samples are available. It should be noted that these substances may not be legally marketable and can be exchanged only in a legal grey area in many countries.

Part 2: Survey of practices — mapping the 'typical' or 'standard' toxicology practices in place in each country

2.1. Objective

The objective of this expert survey was to analyse how the international and national guidance with regard to the post-mortem investigation of suspected DRD cases are translated into practice in Europe (including minimum requirements, recommendations by national expert associations regarding sampling, processing, confirmatory testing, GUS, technical specifications and reporting — prioritising cases of polydrug and mixed poisonings) with special reference to NPS.

2.2. Methods

The identification of experts for the survey was carried out using the following strategies:

- nomination by national focal points and national experts for DRDs from the EMCDDA working group;
- direct communication to state/public institutes for forensic medicine and forensic toxicology departments in universities;
- direct communication with authors of on-topic publications;
- national professional associations.

In total, 118 experts at 95 European laboratories were identified, to whom an online questionnaire (using SurveyMonkey) containing 54 items was sent.

The questionnaire contained questions on the following topics:

- whether or not the participating laboratory undertakes analyses of biological samples in cases of DRDs;
- national or laboratory guidelines regarding general principles of post-mortem toxicological analysis;
- laboratory performance (the number of suspected DRD cases handled per year);
- the laboratory's work as a share of the total national post-mortem analyses;
- the analytical strategy used (pre-test/confirmation; detection/quantification of substances causing death/contributing/additional substances);
- the use of pre-tests by immunological screening/others; substances included in pre-tests and since when these have been included;
- the technical equipment available for quantification of substances, including a retrospective timeline indicating how long they have been used for;
- coverage of substance groups and examples for specific analytes/special drugs such as mescaline, phencyclidine, psilocybin/selected pharmaceutical medicines;
- coverage of NPS, including synthetic cannabinoids, synthetic cathinones, opioids, benzodiazepines, piperazines, phenethylamines, cocaine derivatives;
- standards for toxicological reports (what is included/not included);
- the level of satisfaction regarding the exchange of case-related information between institutions (police/hospitals/forensic pathologists) and forensic laboratories;
- potential hindrances to daily work and analytical advancements;
- an awareness of the laboratory's role as gate-keeper for statistical cause of death registration;
- suggestions for improving the analytical strategy of suspected DRDs.

2.3. Results

2.3.1 Characteristics of participating laboratories

Two thirds (or 63 out of 95) of laboratories from 27 EU Member States, Norway, Turkey and Switzerland responded. Of these, 95 % reported that they perform analyses from biological samples in cases of DRDs, 5 % (n = 3: 2 in Malta and 1 in the United Kingdom) did not. Accordingly, these three laboratories were excluded from the final analysis, as was another laboratory (in Hungary) owing to problems with data transmission. Czechia had a particularly high number of laboratories identified and a very high rate of response. Some of the answers came from laboratories that reported a relatively low annual number of DRD analyses. It was assumed that the national standards of DRD analysis in Czechia are likely to be represented by laboratories with larger numbers of cases, and therefore five Czech laboratories were excluded from the final analysis to reduce the potential for participant bias in the analysis as a result of the over-representation of Czech laboratories. A total of 54 laboratories were used for the final analysis (Table 3).

TABLE 3
Survey response: laboratories per country

Country	Included in final analysis (n)
Austria	1
Belgium	2
Bulgaria	1
Croatia	1
Cyprus	1
Czechia	6 (of 11 laboratories investigating DRD)
Denmark	3
Estonia	1
Finland	1
France	4
Germany	6
Greece	1
Hungary	0 (1 laboratory investigating DRD but problems with data transmission)
Ireland	1
Italy	3
Latvia	1
Lithuania	1
Luxembourg	1
Malta	0 (2 laboratories responded, but were not investigating DRDs)
Netherlands	1
Norway	2
Poland	3
Portugal	1
Romania	0

Slovakia	1
Spain	3
Sweden	1
Switzerland	3
Turkey	1
United Kingdom	3 (4 laboratories responded, one of which was not investigating DRDs)
Total	**54**

With regard to laboratory capacity, 12 % of the laboratories reported that they conduct post-mortem analyses on more than 2 500 cases per year. A total of 13 % reported fewer than 100 cases. Most of the other laboratories carry out fewer than 500 analyses per year (43 %), with results for a further 7 % of laboratories being unknown. With regard to the analysis of suspected DRD cases, 20 % of laboratories reported conducting analyses on more than 250 cases per year and 30 % reported conducting analyses on between 51 and 250 cases. The biggest group, 43 %, responded that they work on fewer than 50 cases per year. This indicates that drug analyses are not a focus of activity in many laboratories; even in post-mortem analyses they are usually a secondary activity.

In response to the question on an estimation of their share of nationwide DRD analyses, 35 % of the participating laboratories indicated that they perform 1-10 % of all cases in the country, 29 % reported that they carry out about 11-50 % of cases, 12 % reported that they carry out 51-95 % of cases and another 12 % reported that they are doing all or nearly all national toxicological investigations for their country (12 % unknown).

In this respect, the study was able to fully reflect the standard in six EU Member States on the basis of the answers of the single responding laboratory that investigates 100 % of the national share of DRDs. These countries are Estonia, Finland, Latvia, Lithuania, Portugal and Sweden. For another six countries (Croatia, Cyprus, Ireland, the Netherlands, Slovakia, Turkey), the one responding laboratory was highly representative of a large number of cases investigated in that country (> 50 % of national share).

2.3.2. Collection of test material for toxicological analysis

Our hypothesis was that there may be variations in how evidence for post-mortem toxicological analysis is gathered. Recent developments in autopsies and post-mortem toxicology techniques may have opened up new options for securing specimens from the human corpse.

In 35 laboratories (65 %), samples for toxicological analyses are always collected from a complete autopsy. However, in 12 countries, some laboratories (see Table 4) reported the use of 'needle autopsies' (minimally invasive procedures for sampling body fluids that waive the need for a full-scale autopsy). The extent to which these procedures are routinely used in individual countries remains unclear. In Hamburg, Germany, it was reported that about 40-60 % of all DRD toxicological analyses are based on blood and urine from 'needle autopsies', but in several other countries the use of this approach was indicated to be rare. It is likely that there is a negative correlation between such minimally invasive sampling and full-scale legal autopsies in suspected DRD cases.

TABLE 4
Laboratories reporting using specimens from 'needle autopsies'

Country	City	Needle autopsy?	Comment
Belgium	Liège	Yes	
Bulgaria	Sofia	Yes	
Czechia	Prague	Yes	
France	Strasbourg	Yes	
	Paris	Yes	
	Grenoble	Yes	
	Versailles	Yes	
Germany	Hamburg	Yes	40-60 % of the DRD cases
	Munich	Yes	Very few
	Dresden	Yes	
Italy	Rome	Yes	
Luxembourg	Luxembourg	Yes	
Netherlands	The Hague	Yes	
Norway	Oslo	Yes	Very few cases
Poland	Lublin	Yes	
Turkey	Istanbul	Yes	
United Kingdom	London	Yes	Blood samples occasionally
	Glasgow	Yes	Rarely, but it does happen with certain pathologists in certain circumstances

The specimens reported as being used in pre-tests (multiple choices) were urine (95 %), serum (39 %), whole blood (50 %) and sometimes other matrices (21 %). Other matrices mentioned included cerebrospinal fluid, vitreous humour, bile, liver, stomach contents, pericardial fluid, muscle and kidney tissues. Apart from cerebrospinal fluid and vitreous humour, other matrices have a major role in cases in which decomposition of the corpse has occurred.

2.3.3. Laboratory equipment

Laboratories in the EU are regularly equipped with — and therefore are supposedly using — modern chromatography equipment, which is usually coupled with MS (Figure 1). Thin-layer chromatography, one of the oldest methods, is still used in 26 % of the laboratories. Various chromatographic techniques for separating analytes coupled with different detectors ('GC coupled' (without GC-MS)

and 'HPLC coupled') are available in about half of the laboratories. Headspace-FID, which uses a special technique for applying samples in a gaseous state to a column for separation and subsequent analysis in a FID, is commonly available (81 %). The equipment most commonly found in laboratories is GC-MS units (87 %); however, liquid chromatography-tandem mass spectrometry units (LC-MS-MS) are also widespread (78 %). Over the past 10-15 years, a shift from GC-MS to LC-MS-MS has been observed due to advances in instrument technology, fuelled by the polarity and low volatility of many new relevant substances and metabolites.

HPLC-TOF-MS, which enables elemental composition to be determined directly, has become the method of choice for GUS in an increasing number of laboratories in recent years and was found in 50 % of the institutions surveyed. The most advanced technical equipment is provided by FTICR-MS, LC-HRMS and UHPLC-HRMS devices, but only two participants in the study had them available. High-resolution mass spectrometry (HRMS) allows mass measurement with high resolution and very high accuracy, while UHPLC, combined with TOF-MS allows additional selectivity, sensitivity and speed resulting from increased chromatographic resolution.

FIGURE 1

Laboratory equipment for analytical oriented chromatography and mass spectrometry (percentage of laboratories equipped)
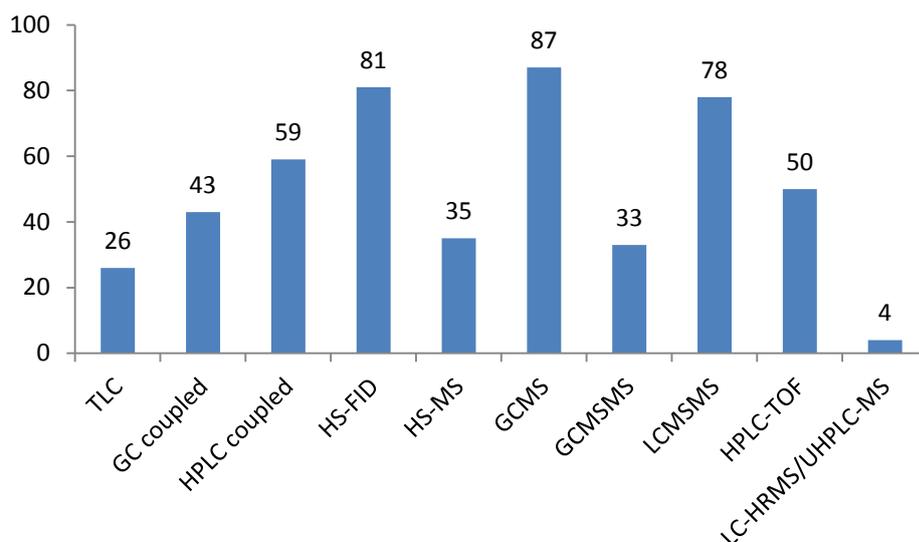
Laboratories equipped with GC-MS and HS-FID, the most common types of equipment, have generally had these available for more than 10 years. The introduction of HPLC-TOF equipment, now reported in half of laboratories, is more recent and has occurred in the past decade, often in the past 5 years. Technical upgrading with LC and GC tandem MS is also a recent development in the majority of laboratories now equipped with it (Figure 2).

FIGURE 2

Time of implementation of new techniques/methods (percentage of laboratories, unknown excluded)
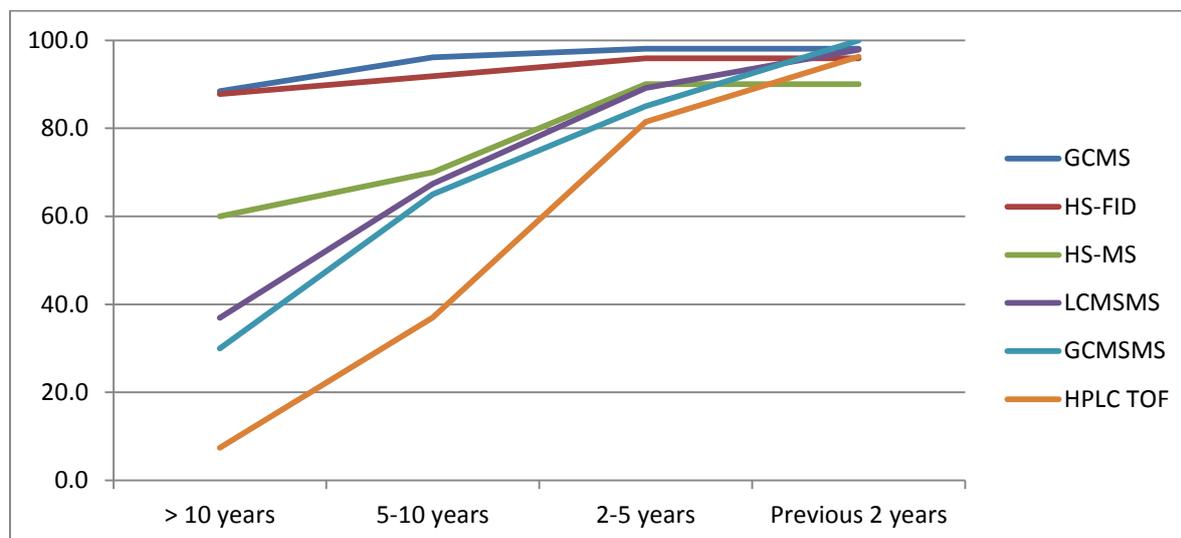
2.3.4. Analytical strategy

All the responding laboratories reported that the objective of their analytical strategy in cases of poisonings is the detection and quantification of the drug or drugs that was/were a direct cause of death. However, 81.5 % of laboratories confirmed that the analysis for *further* (additional/concomitant) drugs/substances, which are not relevant to death, is not the objective of their strategy. This means that in almost 20 % of the surveyed laboratories the completeness of the confirmation of all detectable substances is not a priority. It can also mean that at least 20 % of the laboratories will decide step by step which substance indications in pre-tests will be followed up and which will not. As a result, there may be substances that remain unconfirmed and thus unreported.

It is routinely an option to limit analytics in certain cases. In addition, a laboratory might decide not to determine certain substance groups (in the NPS groups) at all, since the effort required for the validation of appropriate methods involved seems too high or the appropriate techniques may not be readily available. The laboratories reported experiencing a variety of limitations with respect to the toxicological analyses in DRD cases. One third (33 %) reported budgetary reasons resulting in limitations, while a similar proportion (35 %) mentioned shortcomings regarding their laboratory equipment. The most frequent problem reported was the insufficient availability of reference standards (43 %).

The laboratories were asked if they apply conventional pre-tests (screening tests). Immunochemical and chromatographic tests were specified in the question, but there was also an option to provide free-text information on further test principles. A conventional laboratory strategy, which has been available for several decades, is the application of immunoassays, which enable a two-stage analytical approach. If a substance is detected in pre-tests, the findings are confirmed by a confirmatory test. This currently still common practice was affirmed by 70 % of the laboratories. Almost all reported that they had been using these tests for more than 10 years, with only one laboratory reporting their introduction 5-10 years ago. Some laboratories reported immunochemical tests being carried out outside the laboratory by forensic pathologists or coroners in order to have an indicative result at autopsy stage.

By contrast, the question on chromatographic pre-tests may not have been sufficiently precise. Almost all the laboratories confirmed their use; however, it is possible that failing to draw a distinction

between chromatographic pre-tests and the use of current high-performance chromatographic analyses for 'comprehensive screenings' may have posed a problem. These comprehensive screening analyses should not be regarded as a conventional 'pre-test', as they rather have the character of a qualitative confirmation, even if quantification is a further step. In fact, many laboratories use different chromatographic systems for screening and quantification/confirmation of positive immunoassay results depending on the analyte.

Figure 3 shows how often specific substance groups are tested for by those laboratories that indicated their use of immunological screening or other pre- tests. If a specific substance group is not tested for, this may mean that without GUS even in further confirmation analysis this substance group will not be found, even if it is contained in the sample.

Natural cannabinoids, cocaine and opiates are nearly always included in routine screening (one laboratory used immunological screening for natural cannabinoids only and further relied on GUS). Only a few laboratories do not pre-test for amphetamines (5 %) and benzodiazepines (10 %). 3,4-Methylenedioxymethamphetamine (MDMA) screening by immunoassay is performed in 74 % of the laboratories. The use of methadone (66 %) and buprenorphine (58 %) screening is limited and may depend on the probability of occurrence according to substitution treatment practices in individual Member States.

There are other tests that are rarely used, such as those for lysergic acid diethylamide (LSD), but LSD is not relevant for determining the cause of death in poisonings. Fentanyl and oxycodone are rarely included in a pre-test, although they may have a role in the cause of death. For substances GHB, screening tests have not been available for very long. It should be noted that many laboratories that affirm that they use immunological screenings for these groups of substances have established GUS procedures at the same time, so that a double strategy enhances the probability of detection.

FIGURE 3
Substance groups that are at present included in immunological screening (percentage of laboratories with positive answer)
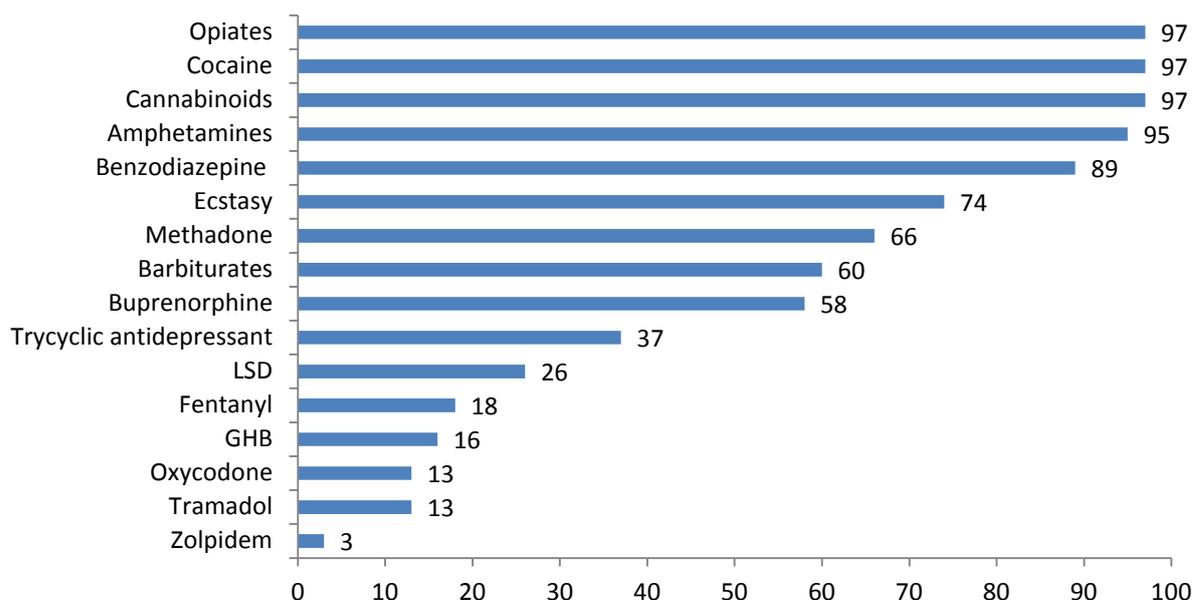
The vast majority of laboratories have not changed their immunoassay pre-testing programme in recent years; individual substance groups were reported as having been added to the programme by less than 10 % of the participants (Figure 4).

FIGURE 4
Substance groups included in immunological screening in the past 5 years (percentage of laboratories with positive answer)

Substance groups recently included in immuno-screening in last 5 years
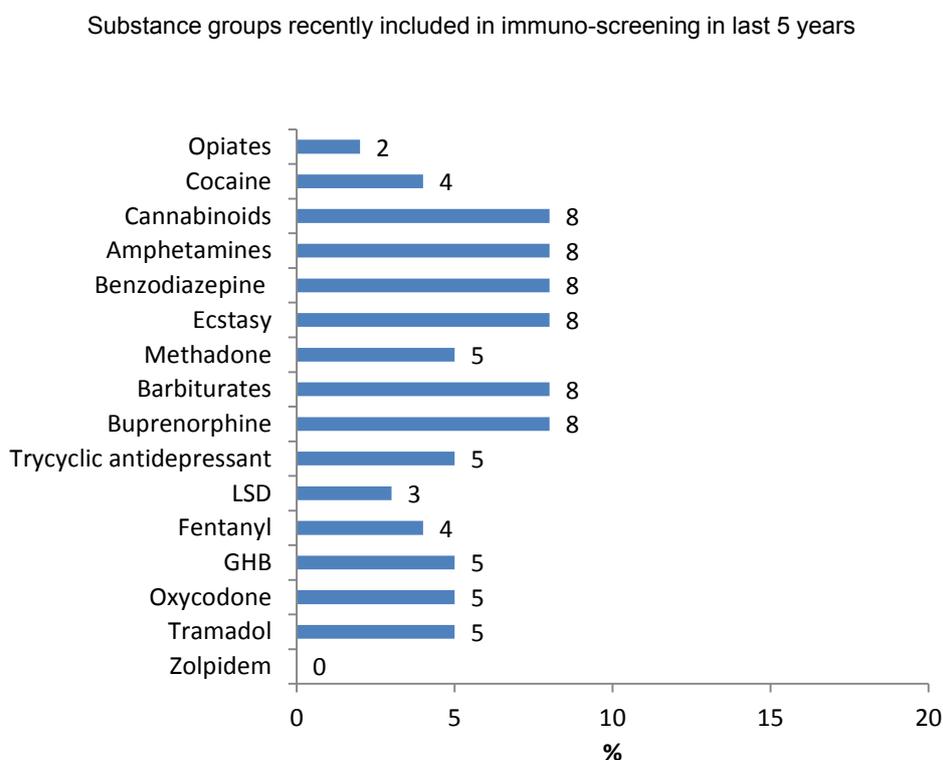
Nearly all laboratories reported that they routinely confirm positive screening test results, with only one laboratory indicating that this decision was made on a case-by-case basis (a laboratory with < 10 DRD cases per year).

2.3.5. Analytical strategy: classification

The results indicate that there are four different analytical strategies (Table 5), mainly due to the differences in the technical equipment and thus the established methods used in the laboratories:

1. A *comprehensive screening* in the strictest sense of a GUS strategy is applied for analysis in suspicious DRD cases in 26 % of all laboratories (n = 14). These laboratories prefer a systematic screening for all substances that can be detected on the available devices, regardless of the case. They use chromatographic multi-target methods applied on LC-MS-MS, HPLC-TOF-MS, UHPLC-HRMS or FTICR-MS. These devices enable the qualitative detection of thousands of substances in a first run, followed by confirmatory analyses with quantitative measurement, using essentially the same methods.
2. In 12 out of the 14 laboratories, the routine screening procedure was performed with a Q-TOF-MS and the quantification with GC-MS, HPLC or LC-MS-MS, depending on the analyte/substance, the case and the circumstances of the death. These 12 laboratories reported using GUS systematically in all cases. However, 3 out of 12 laboratories stated that they still make case-dependent decisions (in addition to a primary GUS), so that it is not always a rigid system. A case dependency could refer to sample properties, to the documented survival time and other factors. The remaining 2 of the 14 laboratories with high-

quality equipment essentially use case-dependent analysis strategies instead of a standard GUS.

3. *Mixed methods*: the questionnaire form did not allow the laboratories to differentiate their strategy for every typical situation in suspected DRDs. On the basis of their report on pre-test screening, GUS and type of available devices, however, it can be concluded that about 43 % of the laboratories (*n* = 23) use advanced technologies such as LC-MS-MS, HPLC-TOF-MS and UHPLC-HRMS for screening followed by quantification, but they still combine it with conventional immunoassays. This situation is typical for laboratories that are not (or not yet) in a position to be certain that their GUS analysis makes immunoassays completely dispensable.

4. Alternative options for the laboratories are to have a limited GUS coupled with either chromatographic methods such as GC-MS or with immunological screening methods. This strategy is combined with analytical decisions depending on the available case information and may proceed with individual selective analyses with different methods. Limited GUS combined with immunoassays was reported by 9 % (*n* = 5) of the laboratories.

A conventional two-step strategy, relying on immunoassays as the first step, was reported by 17 % (*n* = 9) of the laboratories.

Approximately 6 % (*n* = 3) of the laboratories could not be unambiguously classified on the basis of their data.

TABLE 5
Classification of analytical strategies for screening and confirmation in the event of a suspected DRD

Group	Classification	n	%
1	Comprehensive screening with multi-target chromatography screening, including LC-MS-MS, HPLC-TOF-MS, UHPLC-HRMS or FTICR-MS	14	26
2	Mixed methods: immunological screening AND multi-target chromatography screening, including LC-MS-MS, HPLC-TOF-MS or UHPLC-HRMS	23	43
3	Primarily targeted immunological screening or screening based on GC-MS/HS-FID, confirmed by coupled chromatography/MS methods	5	9
4	Primarily targeted immunological screening, confirmed by coupled chromatography/MS methods	9	17
5	Others	3	6
	Total	**54**	**100**

2.3.6. Special analytical considerations and reporting

Two thirds (67 %) of laboratories confirmed that they send substances to a specialised laboratory when they are not able to analyse them themselves (28 % responded 'no'; the situation was unknown for 5 % of laboratories). However, the expectation that laboratories with limited technological equipment/methods will send samples to other laboratories more frequently than laboratories with advanced technology was not confirmed. Rather, fewer than half of the laboratories in the first group reported being able to send samples to a specialist laboratory, compared with three quarters in the other group, which reported having more advanced technology available (Figure 5).

FIGURE 5
'When questions arise about substances you cannot analyse in your laboratory, do you have the opportunity to send samples to a specialised laboratory?' (%)

Laboratories with conventional technology (*n*=14)

Laboratories with advanced technology (*n*=37)
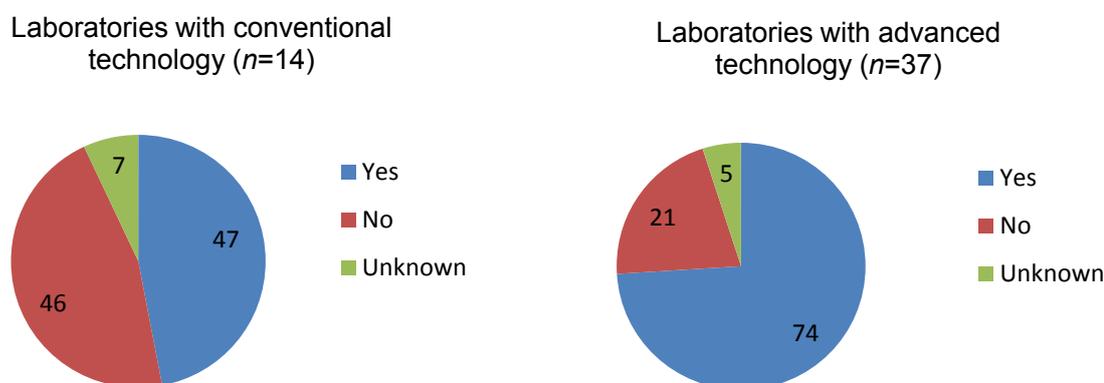
Almost all of the participating laboratories (93 %) indicated that they performed a quantitative determination of the major drug(s) that caused death. Eighty-seven per cent also performed a quantitative determination of additional drugs that might have contributed to death, but only 65 % reported undertaking a quantitative determination of additional findings (e.g. natural cannabinoids). Therefore, there are still some laboratories that base their key findings, at least in part, on qualitative evidence. A clear correlation with the analytical methodology used was not found for this item. However, all laboratories equipped with HPLC-TOF-MS quantified both the primary causes of death and the additional potential contributors to the cause of death.

Quantitative results are included in the final report by only 71 % of laboratories. Two laboratories never or 'rarely' give quantitative statements. An interpretation of the results is not regularly given by the toxicologist in the final report: 77 % of the laboratories with advanced technologies, but only 33 % of the laboratories with conventional methods, provide this aid to interpretation.

Incidental or additional findings that are rated as being irrelevant to the cause of death are rarely listed in the report. Even in the case of laboratories with advanced technologies, only 57 % include this sort of information, meaning that these substances may not be included in any further processing for registration/codification of cause of death or epidemiological surveillance.

2.3.7. Substance group coverage

Opiates, cocaine, amphetamines, methamphetamine, MDA, MDMA, MDEA and the widely used prescription benzodiazepines are practically fully covered by any *routine* analytical strategy being reported by more than 95 % of all laboratories who provided a response for this item. The coverage of natural cannabinoids (93 %) was slightly lower (Figure 6). This was also the case for the prescription opioids, methadone and tramadol (93 % each), as well as for the anaesthetic ketamine (94 %). Drugs that are routinely determined slightly less often are the prescription opioids buprenorphine and fentanyl, the anticonvulsant pregabalin and 'z-drugs' (including the hypnotics zopiclone, zolpidem, zaleplon), with coverage rates of around 70 %. Finally, routine testing for the psychoactive neurotransmitter drug GHB/GBL, volatiles, gases such as butane and propane and 'poppers'(alkyl nitrites, tested for by only one laboratory) are even less common (Figures 7 and 8).

Even *on request*, many of these substances are not tested for in a considerable proportion of laboratories (e.g. testing for buprenorphine either routinely or on request is not available in 9 % of the laboratories; for fentanyl the figure is 4 %, for tilidin it is 46 %, for gases it is 26 %, for volatiles it is 39 %, for 'poppers' it is 41 %, for pregabaline it is 33 % and for GHB it is 13 %).

FIGURE 6

Analytical strategy and capacity for established substance group: coverage in routine versus 'on request' testing (percentage of laboratories)
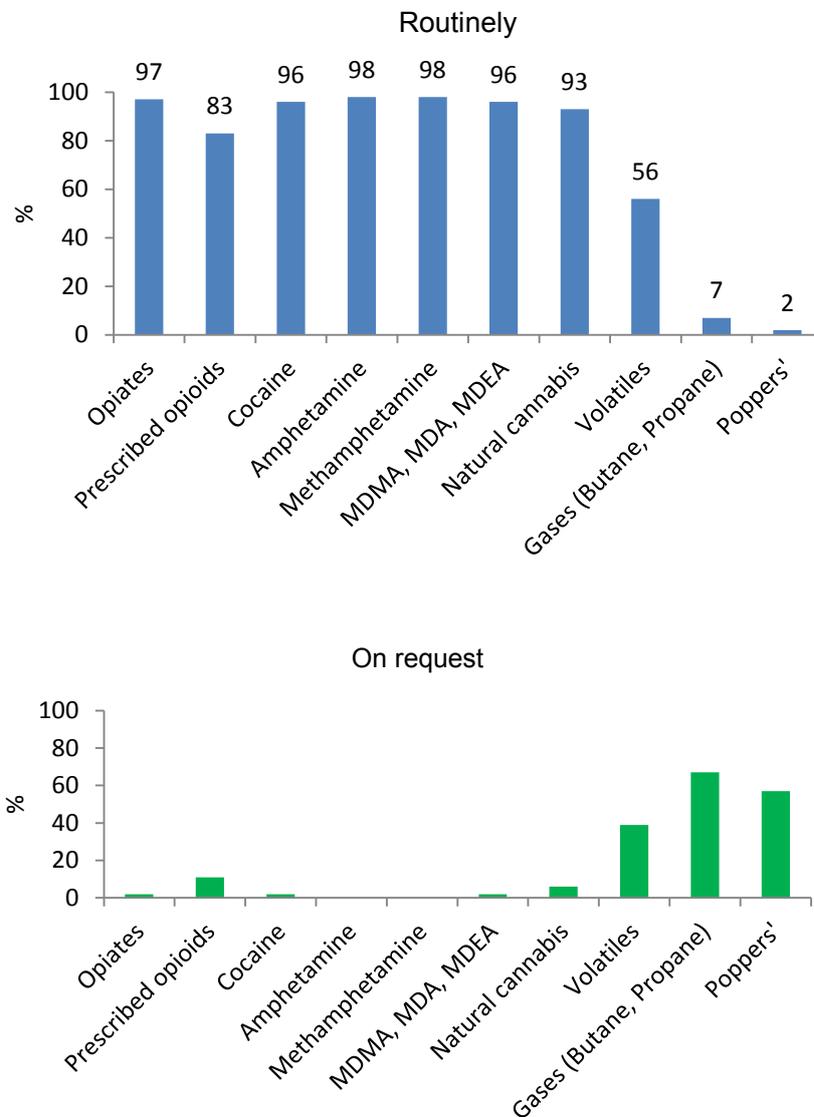
FIGURE 7

Analytical strategy and capacity for some exemplary opioids and other substances: coverage in routine versus 'on request' testing (percentage of laboratories)

Routinely

On request
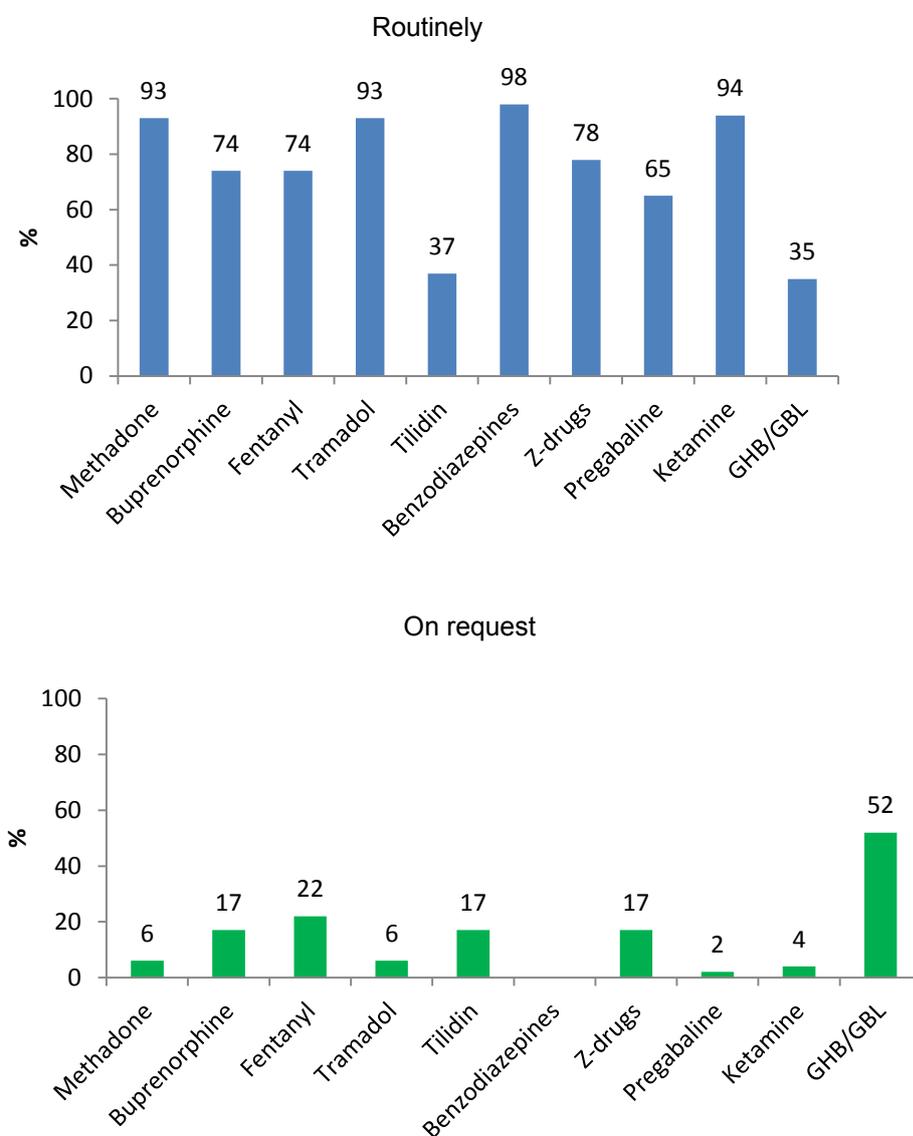
Regardless of the case in question, laboratories with a routine GUS analyse more systematically for substances or groups of substances. The difference between buprenorphine and fentanyl is particularly striking: whereas 92 % and 100 %, respectively, of the 12 laboratories with routine GUS examine these opioids systematically in each sample, the remainder do so in only 69 % and 67 % of laboratories, respectively. Even with pregabalin, GHB and 'z-drugs', the methodological coverage for these substances is considerably lower in laboratories without comprehensive GUS (Figure 8).

FIGURE 8

'Routine analytical strategy covers…': laboratories with (*n* = 12) versus laboratories without (*n* = 42) routine GUS

Laboratories with routine GUS

Laboratories without routine GUS
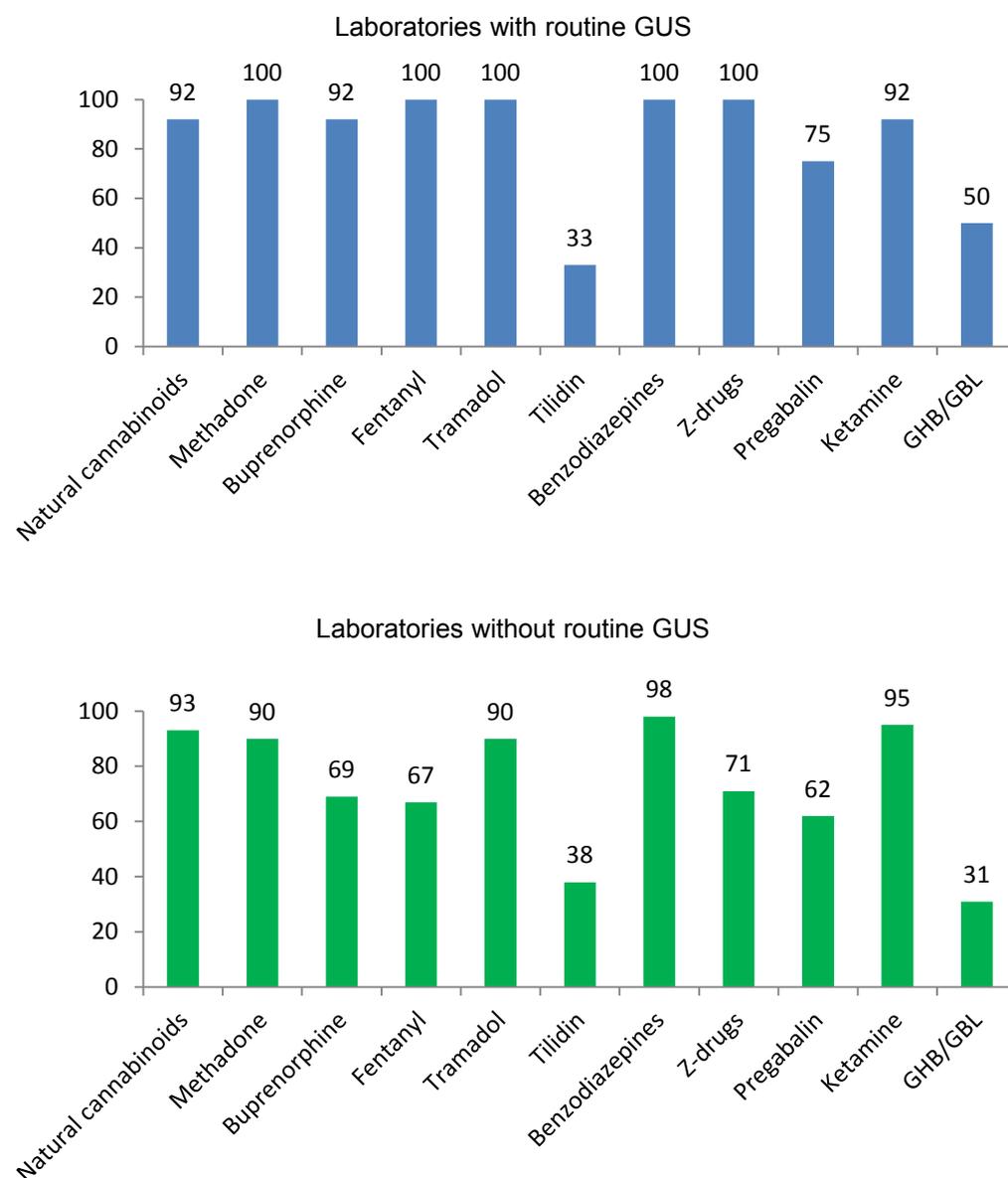
2.3.8. Collaboration/information exchange

When asked about their level of satisfaction regarding the exchange of case-related information between institutions (police/hospitals/forensic pathologists) and their laboratory, only 50 % of the respondents stated that they were 'satisfied' or 'very satisfied', while 21 % of respondents expressed dissatisfaction (Figure 9).

FIGURE 9

Level of satisfaction regarding the exchange of case-related information between institutions
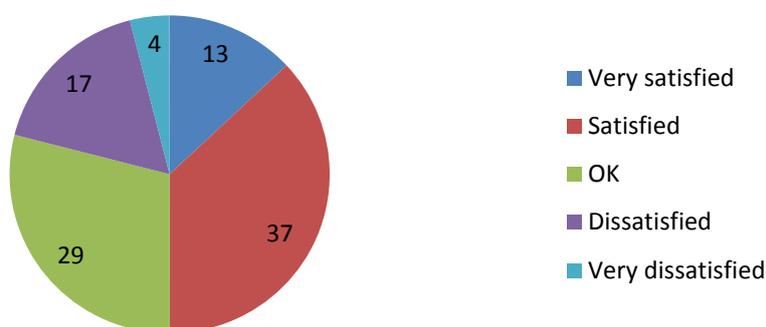
Over half (54 %) of the laboratories were aware that the results of their toxicology reports are used to code causes of death (27 % not sure, 19 % not aware). However, a smaller proportion of participants knew whether the toxicological findings they produced were sent to a General Mortality Register or a comparable institution for the coding of causes of death according to the *International Classification of Diseases*, Tenth Edition criteria (35 % 'yes'; 33 % 'not sure'; 26 % 'no'; 6 % unknown).

2.3.9. Coverage of new psychoactive substances

Among NSP, synthetic cathinones and phenethylamines show the highest coverage in routine diagnostics, in 81 % and 74 % of the laboratories, respectively (Figure 10). Synthetic cannabinoids and piperazines follow, with 65 % each. Synthetic opioids (e.g. new fentanyls), benzodiazepine and, in particular, cocaine derivatives are less systematically investigated routinely.

FIGURE 10

Analytical coverage of NPS in EU Member State laboratories
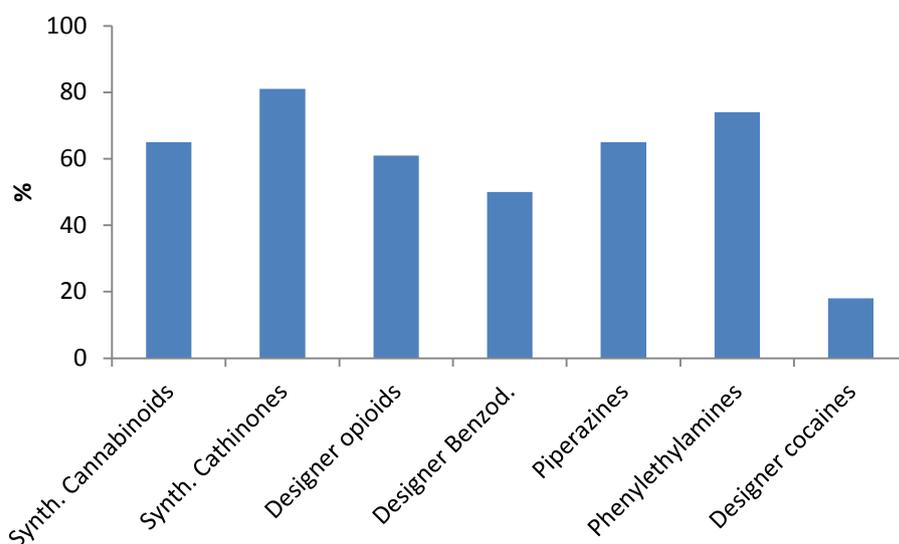
In laboratories with comprehensive screening and mixed methods, almost all NPS groups are examined more systematically and independently of case information than in laboratories with a strategy based on more conventional methods (Figure 11).

FIGURE 11
Analytical coverage of NPS according to laboratory classification in Table 5 (laboratories with advanced multi-target methods = groups 1 and 2; laboratories with conventional methods = groups 3 and 4) (percentage of laboratories with positive answer)

Laboratories using conventional methods

Laboratories using advanced multi-target methods
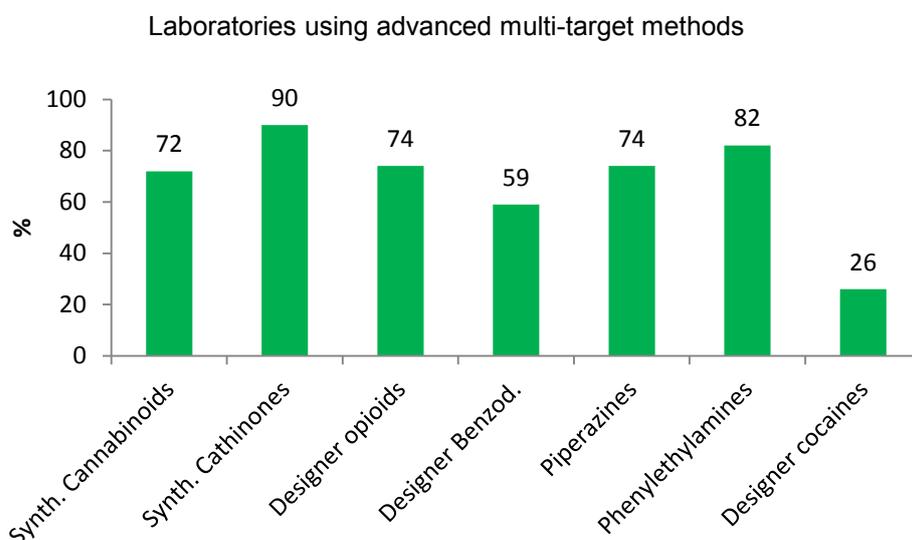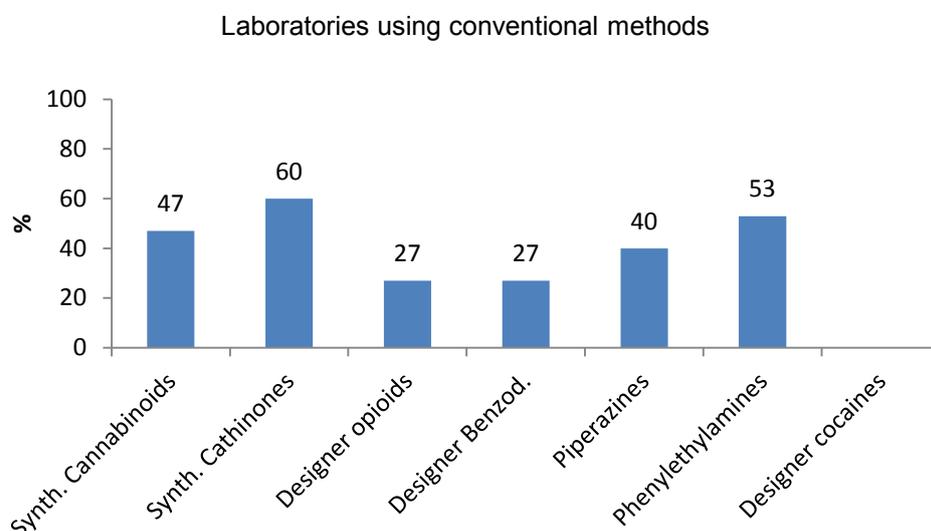
In response to a question about the capacity for analysis of specific substances that exemplify the NPS substance groups included in the previous questions, lower coverage rates were reported than indicated for the groups as a whole. Most of the individual substances had reported coverage rates of between 40 % and 70 % (Table 6).

TABLE 6
'What kind of substances does your analytical strategy usually cover?' (examples)

Substance group	Substance example	Coverage? 'Yes' (% of laboratories)
Synthetic cannabinoids	JWH-18	59.3
	AKB48 Apinaca	37.0
Synthetic cathinones	Mephedrone	81.5
	Methylone	70.4
	Pentedrone	50.0
Designer opioids	O-Desmethyltramadol	64.8
	3-Methyl-fentanyl	44.4
	Acryloylfentanyl	27.8
Designer benzodiazepines	Flubromazepam	40.7
	Clonazolam	40.7
Piperazines	mCPP	68.5
	BZP	63.0
Phenethylamines	2CB	70.4
Synthetic derivative of cocaine	Dimethocaine	14.8
	3-pFBT	9.3
Hallucinogens	Phencyclidine	63.0
	Mescaline	46.3
	Psilocybin	44.4

2.3.10. Suggestions for improvements

When asked for suggestions to improve the analysis strategy for suspected cases of DRD, several participants mentioned the following points:

- networking/communication with other stakeholders (police, justice, health system/clinical doctors);
- cooperation with forensic pathologists;
- standardisation of sample collection;
- with regard to technical and operational needs: equipment, methods needed;
- funding for method development and research;
- guidelines for post-mortem toxicological analyses;
- guidelines for coding cause of death on death certificate.

Box 5 **Limitations of the survey**

The survey gives a rough overview of the state of the forensic post-mortem toxicology field among EU Member States, Norway and Turkey as well as Switzerland. The data collection covered almost exclusively public institutions such as university, state or police laboratories, but it seems likely that post-mortem analyses are performed predominantly in the public sector. The representativeness of the results may be questionable, in particular in large EU Member States such as Spain, France, Italy and the United Kingdom, where post-mortem analyses are shared among quite a large number of laboratories.

Detailed survey questions were generally limited to broad substance groupings, which were considered as a group. It was not feasible within the scope of this survey to collect details on specific analytical methods for particular substances of interest or to gather analytical threshold levels (LOD, LOQ); indeed, the different detection methods reported already reveal dimensional differences in sensitivity. The present study could not describe distinct detailed timelines (e.g. for the past decade), in terms of the development of new methods on new devices for individual laboratories. Hence, a breakdown of new trends in the incidence of certain DRD substances in relation to technical innovations in the laboratories in place remains preliminary at the moment.

Finally, the survey provided evidence, but did not query the causes, for the apparent dissatisfaction regarding inter-sectoral communication (between coroners/forensic pathologists, police, health institutions). Future work should analyse in more detail whether, in individual countries/between individual regional actors, the

exchange of information as provided by regulations remains insufficient or whether there are fundamental obstacles, such as privacy policy and interface issues (e.g. between public authorities, university research laboratories or independent laboratories).

3. Discussion and conclusions

3.1 Interpreting drug-related death prevalence data against the background of toxicology standards and capacity in European countries

The implementation of new methods and technology in the laboratories yields more results for substances that are missed by traditional analytical strategies. These include new substances that may cause harm owing to their chemical and pharmacological properties and/or to their high potency, which may cause hazardous health effects at very low concentrations. Changing from one method or standard to another will influence trends.

The principle 'the more you search, the more you find' clearly has an impact on DRD monitoring at regional or country level. This has been shown for Sweden, where the number of detected fentanyl cases doubled when there was a switch from the earlier occasion-related testing for drugs to comprehensive ('full') screening on every forensically investigated death in September 2011 (Leifman, 2016). The implementation of new technology allowed for lower LODs in a centralised forensic laboratory for post-mortem specimens from all over the country. A technical upgrade of laboratory methods — which could be synchronised at a national level — offers the opportunity for the retrospective analysis of stored samples. Such local studies could provide insight into the shift of analytical (and monitoring) sensitivity for some opioids and more particularly for NPS, for example.

The survey reported here showed that HPLC-TOF mass spectrometers have been used in some laboratories in Helsinki, Finland, Copenhagen, Denmark, and Frankfurt, Germany, for more than 10 years; in laboratories responding from other countries, the investment in this technology was made less than 10 years ago. Laboratories in Nicosia, Cyprus, Berlin, Germany and Birmingham, United Kingdom, recently put the technology into operation (less than 2, years ago). In many other laboratories, the technical upgrade to an HPLC-TOF mass spectrometer took place 2-5 years ago. Among these laboratories, those in Istanbul, Turkey, Oslo and Trondheim, Norway, and Vilnius, Lithuania, are in the position of handling a majority or nearly all DRD cases in their countries. Therefore, in these countries, a trend analysis of deaths related to synthetic opioids and NPS in the 4-year period from 2013 to 2016 could be a promising way to analyse a potential monitoring artefact. Importantly, in the other countries, where the responding laboratories handle only part of all DRD cases, generalisation of the findings to the country level should be avoided.

However, interpreting trends will be challenging and consideration will need to be given to a range of potentially confounding factors and interactions, including those between changes in patterns of use and the influence of various regulatory and health policy measures on both use of substances and the availability of the equipment for new toxicological investigations and the extent to which these are implemented. The pattern of polydrug poisonings will require an in-depth analysis in these observational studies, as some illicit substances that were previously hidden but that accompanied other, easier to detect, substances may become visible.

3.2. Conclusions

In Europe, the majority of DRDs are registered with a 'known toxicology' in the EMCDDA Statistical Bulletin, but there is only fragmentary knowledge of limitations and regional or national differences in the analytical capacity of forensic toxicology laboratories in terms of routine substance screening. While rapidly evolving technologies have resulted in an increasing number and range of toxicological assessment methods being available, the diversity of substances that are potentially involved in deaths means that there is not a single method that can cover them all (at least with adequate LODs).

Importantly, in many countries in Europe, post-mortem forensic toxicology is restricted to a single or small number of laboratories, run by public authorities. Hence, any change in standard operating procedures in a single laboratory can potentially have a major influence on DRD prevalence data.

Over the past 10 years, a comprehensive screening approach using advanced HPLC-TOF-MS, FTICR-MS, GC-MS-MS or LC-MS-MS has become increasingly available in forensic laboratories. Such screenings do not cover all possible substances but have become the method of choice for analytes with forensic relevance in very low concentrations in biological specimens.

As there is currently limited experience in the post-mortem analysis of NPS-related poisoning cases, universal analytical recommendations are very difficult to make in this field. In addition, the lack of reference standards leads to great restraint in the reporting of results, even where qualitative determinations are possible.

The survey conducted with a sample of 54 forensic laboratories in Europe showed that the majority of the responding laboratories have shifted to multi-target screening methods, including advanced technologies and instrumentation. A strict GUS that assesses the broadest spectrum of substances currently possible for every incoming case is meanwhile applied in just over one quarter of the participating laboratories. Conventional targeted methods such as immunochemical screening are still common as a first step but are used selectively.

There are still challenges for the reliable determination of highly potent opioids and NPS in laboratories without systematic GUS strategies, which, as indicated, are still not adopted in many countries. Particularly in polydrug poisonings, prescription opioids and other NPS may go undetected/unreported, although they may have caused or contributed to death; the same is true for the coverage of certain non-opioid prescription drugs such as pregabalin. Some other substances that may be misused and result in sudden death, for example solvents and alkyl nitrites ('poppers'), are also challenging to detect in routine sampling and analysis owing to their volatility, and, for the majority of laboratories, their detection needs case-dependent decisions.

In the case of an NPS-related death, a laboratory without adequate equipment for comprehensive screening will struggle to identify cause of death, and further analytical decisions will critically depend on the quality of the case information. Nonetheless, even if police information actually gives specific indications on the use of NPS before death, in many cases it will be necessary to send specimens to a specialised laboratory. However, this study indicates that, for the survey respondents, this option is not always available, particularly for those laboratories without advanced technical equipment. In addition, the potential to send specimens away for analysis may be limited owing to sample volumes, storage and transport conditions (and funding).

3.2.1. Consequences and priorities for public health

One of the important conclusions of this review is that specific medico-legal recommendations for decision-making on ordering toxicological examinations after autopsies would be valuable. Such recommendations should include guidance for ordering and conducting examinations on all cases of young/middle age unexpected deaths or with equivocal cause of death identified at autopsy or with a potential natural cause of death whenever a drug may have precipitated or contributed to death by an additive mechanism. Importantly, it has been demonstrated that the threshold level for toxicological investigations has a considerable impact on DRD monitoring and on the quality of substance-related data available. Therefore, guidelines should be instrumental in helping to harmonise these threshold levels.

The report suggests that national guidelines for forensic toxicological laboratory analysis should be reviewed, addressing in particular the following questions: 'Do laboratories analysing biological samples have sufficient facilities, competence and training to contribute to reporting of data relevant

for public health?', 'Is the codification of the toxicological evidence of NPS in deaths sufficiently clear for General Mortality Registries under WHO rules?' and 'Is the integration of the laboratories in early warning networks sufficiently ensured?'.

In the context of rapidly changing laboratory techniques, it is also important to highlight that guidelines often take into account the average technical laboratory instrumentation available at the time they are developed, and thus they should be updated regularly to reflect developments in instruments and analytical approaches. Another important conclusion of this work is that there is a need for an update of national/European/professional society reference documents to include a recommendation that a comprehensive screening strategy based on advanced analytical technology is adopted. That will be useful for DRD casework but also for the epidemiological monitoring of substance use.

For the epidemiological surveillance of NPS, the challenge is that at least qualitative results (i.e. the detection and identification) are reported. Bearing this minimum requirement in mind, owing to the specific challenges associated with the large and rapidly growing number of NPS, it is likely that only selected or centralised laboratories in relatively large Member States will be able to become specialised in analyses for these from biological samples. Indeed, there are special requirements in post-mortem analysis and difficulties in their interpretation, which challenge laboratories with relatively few DRD cases per year.

Therefore, experience and expertise in the determination of hazardous NPS in specialised laboratories should be shared (e.g. by facilitating further updates of mass spectral libraries). This knowledge sharing could entail in particular police laboratories analysing substances from seizures, in collaboration with the laboratories analysing biological samples. Furthermore, this sharing, within countries, could stimulate further European/international collaboration, through a strategically strengthened exchange of information across the relevant EU organisations, the EMCDDA, and the forensic and toxicology organisations (e.g. to provide online access to mass spectra of emerging NPS).

Beyond qualitative analysis, it is important to note that reference standards necessary for the quantitative determination of emerging NPS are available from industrial sources but only after a considerable time delay and at significant cost. To address this issue, the use of high-purity confiscated samples as a reference may solve the problem, but, evidently, this requires the exchange of information about where such samples are available. In this context, the problem arises because the substances are not marketable by law and, consequently, situations can vary across countries. The situation is indeed complicated by the fact that, while in some countries the process is well specified by legal provisions, in others the exchange among laboratories constitutes a legal grey area. In this context, an EU-wide inventory of how to deal with legal grey areas while exchanging seized materials for analytical purposes may increase the security and success of action taken by forensic laboratories. Furthermore, the question of improving national and international data exchange between laboratories on available new substances should be prioritised.

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